ODOR MIXTURE PERCEPTION OF STRAIGHT CHAIN ALDEHYDES C_6-C_{11}

A Thesis

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ABSTRACT

Physiological studies examining the binding properties of the olfactory receptor I-7 (OR-I7) has identified octanal (C_8) as the primary agonist for this receptor. However, the molecular range of the receptor has been shown also bind odorants straight chain aldehydes C_7-C_{10}. Free-choice profiling of odorants C_6-C_{12} identified as striking difference between the qualitative differences of C_6 and odorants C_7-C_{12}. Identifying C_6 as grassy/green and odorants C_7-C_{12} as more citrus in character. An adaptation study of odorants C_6, C_8, C_{10}, and C_{11} was conducted in order to examine the cross-adaptation properties of these odorants and determine whether odorants which do not bind with OR-I7 cross-adapt. Prior studies have shown cross-adaptation in influenced by odorant quality similarity as well as chemical functionality similarity. Furthermore, odorants sharing common receptor sites are known to cause cross-adaptation. It was hypothesized that C_6 would not cross-adapt with odorants C_8, C_{10}, and C_{11}. But the odorants characterized as citrus (C_8, C_{10}, C_{11}) would readily cross-adapt. In order to evaluate changes in intensity due to adaptation, a methodology using odor reference matching was devised. All odorant pairs were tested for cross-adaptation and results determined that C_6 did not cross-adapt with the citrus odorants C_8, C_{10}, and C_{11}.

This project also examined the odor mixture perception and the ability of the individual to detect components within a mixture. There are two theories supporting odor mixture perception. One theory states that the components comprising an odor mixture are detectable; while the other theory suggests that the components within a mixture combine to create a novel odor making the individual components impossible to detect. Odorants with dissimilar odor

qualities are known to be easier to detect within an odor mixture. From the previous study it had been shown that C_6 and C_8 have very different odor qualities and do not cross-adapt. A series of binary odor mixtures of C_6 and C_8 were examined where the ratios of the intensities of each of the components was varied. Subjects were trained by using a reference matching task to identify the intensities of the individual components within the mixtures. Furthermore, subjects were asked to identify a single component within the binary mixture and determine the intensity of that component. Subjects made quick decisions of the perception of the intensities of the components within the mixture through a gestalt. The reference odorant was the figure, the subject was asked to find the figure within the mixture and determine it's intensity. The other odorant present within the mixture was the ground. As hypothesized as the ground odorant increased in intensity, the ability to properly identify the intensity of the figure odorant became increasingly more difficult due to the effects of mixture suppression resulting in figure suppression.

From the first experiment it is understood that C_6 and C_8 do not cross-adapt; however, results from the second experiment suggest that C_6 and C_8 demonstrate the effects of mixture suppression. These results suggest mixture suppression and adaptation must occur at two different stages of odorant processing within the olfactory process.

BIOGRAPHICAL SKETCH

Anne Judith Kurtz was born in New York, New York on August 7, 1983. She graduated from The Dalton School in May 2002. Afterwards, she attended Hamilton College in Clinton, New York where she majored in Chemistry and minored in Psychology. She graduated May 2006, cum laude with a Bachelor's of Art. She was a member and captain of the Hamilton College Women's Crew Team, Psi Chi, Sigma Xi, and received the Elihu Root Fellowship. She also worked for two summer's under the guidance of Dr. Harry Lawless at Cornell University as a sensory technician as an undergraduate at Hamilton College.

Anne began her graduate studies at Cornell University on August 2006. Her study of concentration was flavor chemistry with a minor in sensory evaluation under the direction of Dr. Terry Acree and Dr. Harry Lawless. She presented her research at the annual meetings of the Weurman Symposium July 2, 2008 and the 15th Annual International Symposium on Olfaction and Taste (ISOT/ AChemS) on July 24, 2008. While at Cornell she received the Outstanding Teaching Assistant Award for her work on Food Science 430: Understanding Wine and Beer. She was a member of the product development team, food science club, and Phi Tau Sigma. She is currently a member of AChemS and IFT. Anne will receive her M.S. in August of 2008, and will begin working at Unilever as a fragrance intern in September of 2008 and hopes to return to Cornell for further study.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

OSN: olfactory sensory neuron

OB: olfactory bulb

OR: olfactory receptor

C_6: hexanal

C_7: heptanal

C_8: octanal

C_9 nonanal

C_{10}: decanal

C_{11}: undecanal

C_{12}: dodecanal

aPCX: anterior piriform cortex

OBP: olfactory binding protein

OR-I7: olfactory receptor- I7

GPCRs: G-protein coupled receptors

ACII: adenylyl cyclase

ATP: adenosine triphosphate

cAMP: 3’5’-cyclic adenosine monophosphate

CNG: cyclic nucleotide gated channel

GFP: green fluorescent protein

EOG: electrophysiological recordings

3-AFC: 3-alternative forced choice

LSD: Least Significant Difference

CHAPTER 1
LITERATURE REVIEW

1.1 INTRODUCTION

Olfaction processes odorants of various sizes and chemical functionality into distinct percepts creating a sensory experience. A sensory percept has two orthogonal features: qualia defined as the feeling of the conscious experience such as the smell of an orange (Edelman 2004) and the intensity or strength of the experience. Psychophysics allows researchers to measure the intensity of the qualia produced by different stimuli, thus translating perceptions into quantifiable units (Gescheider 1997). It is not well understood what brain processes convert an odor into an experience especially when the odor comes from a complex mixture of stimuli. However, there are two dominant theories describing mixture perception: the configural and the elemental. The configural, combinatorial or synthetic theory states that individual odor components are synthesized into new and novel odors not produced by the components of the mixture (Malnic et al 1999). The elemental or analytic theory argues that mixtures are comprised of components, where only the components are able to be detected in the mixture (Kay et al 2005, Kay et al 2003, Laing 1986, Laing et al 1994, Laing & Francis 1989). Clearly, understanding how individuals process odor mixtures is crucial to understanding the relationship between odor chemistry and odor perception.

1.2 OLFACTION

Vision and audition are determined by and limited to specific wavelengths of light and sound. Similarly, the olfactory system is capable of detecting only certain chemicals, all less than 300 Daltons (http://www.flavornet.org (Arn 1998) with a composition and structure able to stimulate olfactory receptors (ORs) in the olfactory epithelium (OE). Also, an odorant's volatility above a food or fragrance matrix further limits its potential to contribute to odor perception. Volatility as illustrated by Henry's Law (equation 1.1) relates solubility and vapor pressure of an odorants to its ability to reach the OE in sufficient concentration to activate any ORs.

$$P_B = k_B X_B$$

The solute vapor pressure (P_B) is proportional to the solute mole fraction (X_B) and Henry's law constant (k_B) for the solute B in solvent A (Tinoco 1995). Thus gas solubility is directly proportional to pressure, dependent upon the temperature and the nature of the matrix. In order for an individual to detect flavors from food or fragrances, the volatiles must first partition from the food or fragrance into the air and travel from the air into the water-mucous covering the OE in the nasal cavity. Odorants must posses a particular volatility, stability, solubility, reactivity and access to the OE in order to effectively activate ORs (Mozell 1970); (Firestein 2001) and produce a qualia.

Although it has been reported that mammals are capable of detecting more than 10,000 odorants, in nature these perceptions are likely caused by less than 1,000 odorants (Axel 1995); (Arn 1998). In order for an odorant to be perceived, the odorant must be dissolved in the OE located in the upper nasal cavity below the brain, where odorants interact with cilia containing specific ORs. An odorant is capable of reaching the OE via two different routes: either the orthonasal or retronasal pathways illustrated in Figure 1.1. Orthonasal olfaction occurs when odorants enter the nasal cavity through the nose where it reaches the nasal epithelium and interacts with ORs on the epithelium.

Retronasal olfaction ensues when odorant molecules released by food into the oral cavity and nasal pharynx travel through the nasal pharynx into lungs and then back to the nasal cavity, where they interact with the OE. Once an odorant enters the nasal cavity and binds to an OR it starts a cascade of electrical signals that is translated into information and experience by the brain.

A series of citrus smelling straight chain aliphatic aldehydes whose carbon backbone ranges in length from 7-10 carbons has been shown to excite the olfactory receptor I7 (OR-I7) (Zhao et al 1998) and octanal (C_8) has been identified as the primary agonist for OR-I7. In an attempt to understand how odor binding and processing produce an intensity and distinct qualia, this thesis will contrast and compare the psychophysics of the "citrus" smelling OR-I7 agonists with hexanal a "green" smelling inhibitor (in rat preparations) of the OR-I7 receptor.

1.3 OLFACTORY ANATOMY

Once an odorant dissolves in the mucosal lining of the nasal epithelium and binds to an OR, an electrical signal is transduced in olfactory sensory neurons (OSN), up into the olfactory bulb (OB). OSNs each express a unique olfactory receptor (OR) that determines the exact spot in the OB where it terminates. The surface area of the nasal epithelium is estimated to vary among individuals from 1-5 cm^2 (Morrison & Costanzo 1990). Only one type of OR is expressed by an OSN (Chess et al 1994, Malnic et al 1999, Mombaerts 1999, Mombaerts et al 1996, Rawson et al 2000, Serizawa et al 2003) but a

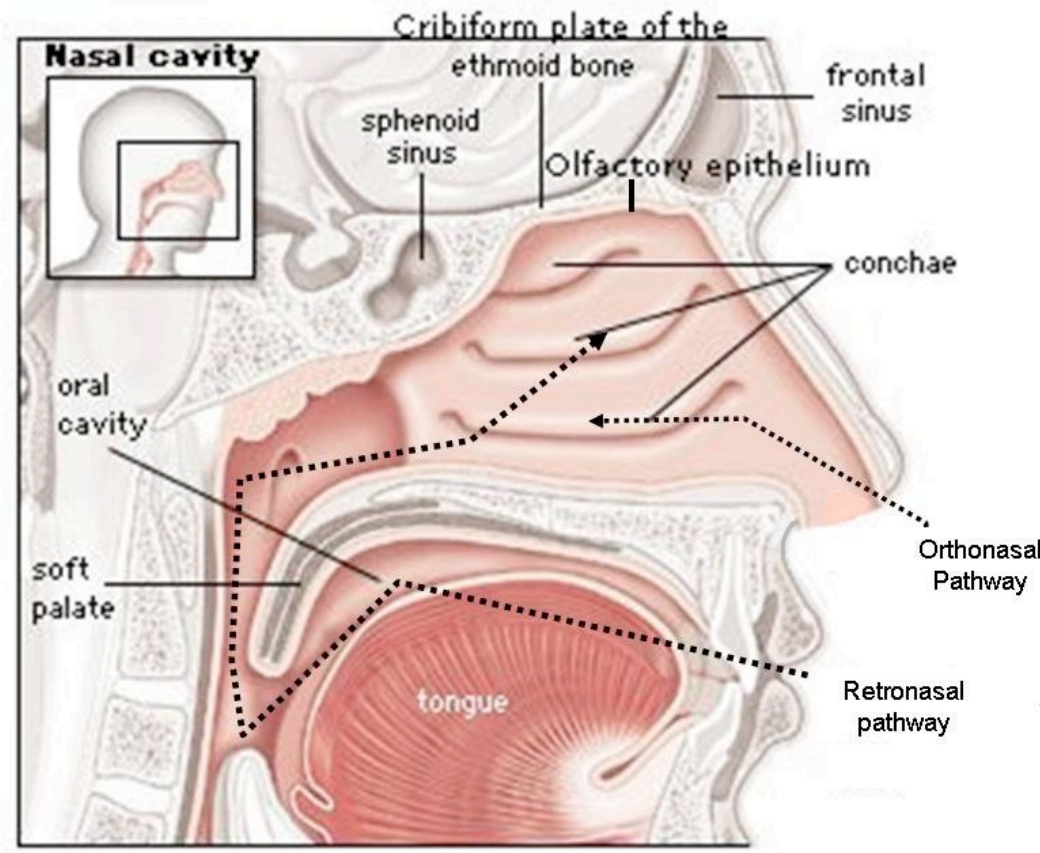


Figure 1.1: Orthonasal and retronasal pathways illustrated by dashed lines. The location of the olfactory epithelium and cribiform plate. Modified from the encylopedia britannica online.

single chemical (ligand) is capable of activating more than one OR thus increasing the chance that all olfactory receptors can be stimulated by a limited set of odorants, (Araneda et al 2000, Duchamp-Viret et al 1999, Singer 2000). The ability of odorants to bind to more than one type of receptor is illustrated by the concept of molecular range (Araneda et al 2000). The axons of OSNs expressing the same receptor protein converge at the glomerulus located in the OB ((Mombaerts 1999, Paysan & Breer 2001). Dendrites of both mitral and tufted cells are located inside of a single glomerulus. These cells transmit olfactory information onto the cortex for further processing (Kandel et al 2000).

In order to protect the olfactory system from damage, air passing through the nose is humidified and warmed. The mucus layer lining the olfactory epithelium acts a protective layer, trapping dirt and other foreign substances from entering the body further. The mucus is excreted from Bowman's glands through ducts located beneath the OE and open onto the surface of the epithelium (Kandel et al 2000). The aqueous mucus contains immunoglobins and olfactory binding proteins. As the odorant diffuses through the mucus layer of the OE it binds to olfactory binding proteins (OBPs) (Matarazzo et al 2002, Pevsner et al 1988a, Pevsner et al 1988b) whose function is still unknown.

The olfactory epithelium contains 6-10 million OSNs, which are interspersed with glial-like stem cells. Both OSNs and the supporting cells (sustenacular) are located above the basal stem cells and are found at the base of the epithelium. OSNs are unique among neurons due to their short life-span, lasting 30-60 days. OSNs undergo a process called neurogensis, where the neurons die and are replaced continuously. Neurogenesis is particularly beneficial given that the olfactory system is continuously inundated by harmful substances including allergens, pollutants, and microorganisms (Kandel et al 2000). Nerves regenerate from basal cells and replace old OSNs.

OSNs are bipolar cells extending two processes from the cell body. From its apical pole is a single dendrite which extends to the surface of the epithelium ending in a knob-like swelling from which project thin cilia (approximately 5-30) into the mucus layer of the nasal cavity. The ORs are located within the membrane of these cilia. From the basal pole of each neuron, a single axon is projected through the cribiform plate, located above the nasal cavity of the olfactory bulb (Firestein 2001, Kandel et al 2000). The axon forms synapses within the olfactory bulb neurons and relaying signals to several locations in the cortex and the supporting cells release immunoglobulins into the mucus.

Axons of OSNs expressing similar ORs congregate at glomeruli located in a single region of the OB (Mombaerts 1999, Mombaerts et al 1996, Paysan & Breer 2001). Glomeruli, as illustrated in Figure 1.2 are anatomically discrete, these structures are spherical and are constructed of nuerophil 50-100μm in diameter (Firestein 2001). Tufted cells allow for communication between glomeruli containing OSNs expressing the same ORs. Surrounding the glomeruli are periglomerular cell, which provide cross-talk and center-surround inhibition (central groups of neurons suppress the activity of neighboring cells (Aungst 2003, Aungst et al 2003a, Aungst et al 2003b). Granule cells reside within the mitral cell layer. Granule cells lack axons; however, each granule cell contains several spines on its dendrites, which synapse with mitral and tufted cells providing excitatory and inhibitory control.

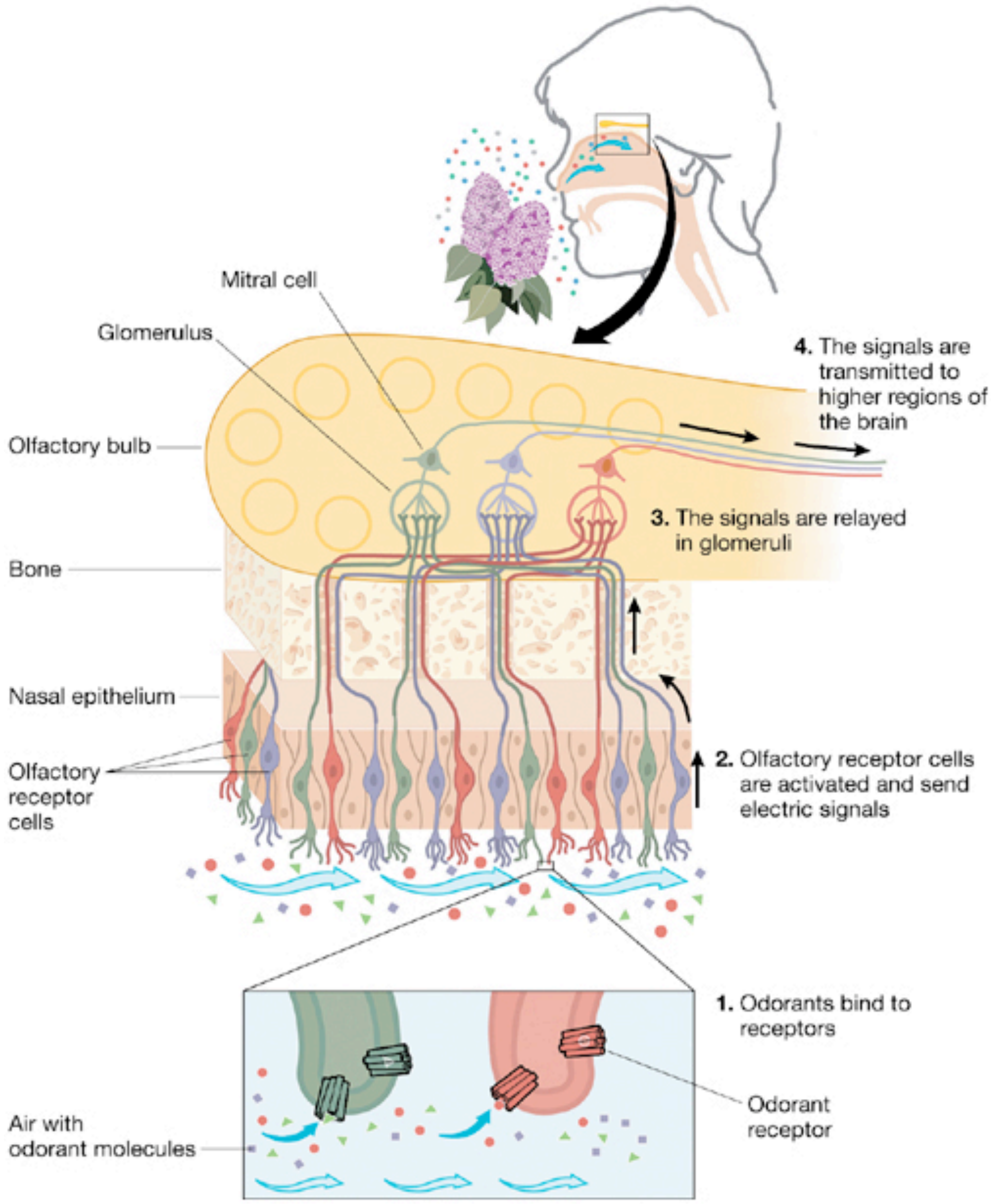


Figure 1.2: Schematic of peripheral olfactory nerve organization. Odorants enter the nasal cavity where they are absorbed into the olfactory epithelium and interact with odorant receptors, initiating the signal cascade. Signals are sent to glomeruli located in the olfactory bulb within the brain where the signal is further transduced onto higher cortical regions. Reprinted by permission of EMBO reports (Rinadli, 2007)

Glomerular studies have shown that images sent from glomeruli in the OB and on into the brain form a topological map for odors (Mori et al 1999). Multiple mitral cells located within each glomerulus act as a convergence pathway. It is estimated that 1,000 neurons converge onto each glomerulus (Hellman and Chess 2002). Each mitral cell extends a single dendrite into the glomeruli. The axons of mitral cells form a lateral olfactory tract which route the olfactory signal from the olfactory bulb to the olfactory cortex, where signals travel to other regions of the brain for further processing.

Unlike most sensory processes, olfactory information does not travel directly to the thalamus for higher brain processing; instead several different brain areas are affected. fMRI studies have served to further elucidate the different areas of the brain which are excited by olfactory stimuli. Some axons project to the piriform cortex, amygdala (an area highly correlated with emotions and sexual behaviors), the hypothalamus, as well as the thalamus and hippocampus (Shepherd 2006, Small 2004, Small et al 2004). The diverse number of brain regions involved in olfactory processing explains why upon smelling a stimulus so many different emotions and experiences are recalled to create an individual perception.

1.4 OLFACTORY RECEPTORS

ORs are G-protein coupled receptors (GPCRs) of which there are nearly 1,000 coding making it the largest family of GPCRs in the mammalian genome. GPCRs have several unique structural features: a coding region lacking introns, seven α-helical membrane-spanning domains connected by intracellular and extracellular loops of variable lengths, as well as several conserved short sequences (Touhara 2008). A unique feature of ORs is a longer second extracellular loops and an extra pair of conserved cysteines contained within the loop. The binding pocket for odorants on the seven transmembrane protein has not been identified yet; however, binding most likely involves helices III, IV, and V (Pilpel & Lancet 1999, Man et. al., 2004).

1.4.1 OLFACTORY TRANSDUCTION

Upon ligand binding to an olfactory receptor GDP is converted to GTP. The G-protein is a heterotrimer. The α subunit dissociates from the β and γ sub-units. The receptor then attaches to a membrane bound adenylyl cyclase (ACII). ACII stimulates the conversion of adenosine triphosphate (ATP) into 3'-5'-cyclic adenosine monophosphate (cAMP). In signal transduction cAMP both activates the opening of ion channels as well as activating protein kinase A (PKA). cAMP stimulates PKA, in the absence of cAMP, PKA remains inactive. PKA is a tetramer, composed of two regulatory subunits (R) and two catalytic subunits (U). cAMP binds to the R subunits of PKA, which releases the two active catalytic subunits. The level of cAMP in this feedback loop determines level of PKA. PKA phosphorylates enzymes, and transfers phosphorous groups of ATP onto other proteins.

cAMP also binds to ion gated channels, capable of conducting Ca^{2+} and Na^{+}, creating an electrical voltage differential when the cyclic nucleotide gated channel (CNG) is open. Resting potential of OSNs is -65 mV, upon opening of the ion gated channels; an influx of Ca^{2+} and Na^{+} creates an increase in positive ions inside of the cell (Firestein 2001). This positive signal propagates along the axon of the OSN, up through the cribiform plate, whereupon the signal synapses with second-order neurons and into the olfactory bulb. Once the GPCRs are activated by the binding of an odorant, the signal is amplified,

Cl^- ions depolarizes the cell. The signal is further depolarized and thus strengthened by the efflux of Cl^- out of the CNG (Firestein 2001). A negative feedback loop is created when Ca^{2+} ions enter CNG channels, leading to an adaptation response. Once the signal reaches the olfactory bulb, olfactory signals are trandsduced along the olfactory nerve and onto several different cortical regions. Signals are sent to the glomerulus, amygdala, orbitofrontal cortex, and the piriform cortex (Firestein 2001).

1.4.2 OLFACTORY RECEPTOR I-7

In 1991 Buck and Axel identified and sequenced 18 different cDNA clones, one of which was the OR-I7 olfactory receptor. In 1999 Zhao et. al. identified the first OR-ligand agonist pair to determine the ligand binding properties of the I7 gene. Zhao et. al, found octanal (C_8) to be the primary agonist for OR-I7 (Zhao et al 1998). Zhao et. al. (1998) noted that of the 74 odorants tested, the straight chain aldehydes, heptanal (C_7), (C_8), nonanal (C_9), and decanal (C_{10}) elicited the greatest electrical response. Table 1.1 illustrates the properties of C_6-C_{11}. Zhao, et. al. (1998) injected rats with an adenovirus vector which co-expressed the rat OR-I7 receptor along with a green fluorescent protein (GFP). Upon infection of the rat olfactory mucosa with the adenovirus, electrophysiological recordings (EOG) were taken as a measurement of excitation (Zhao et al 1998). The summed recordings of the infected neurons indicated the greatest numbers of neurons were excited by exposure to C_8. In order to further confirm these results patch clamp recordings were collected by placing an electrode tip onto a patch of cell membrane, thus making it possible to record the flow of current through the ion channels of individual cells. The recordings revealed that OR-I7 displayed the greatest response to C_8, by producing the greatest electrical current. The modified data in Figure 1.3 is plotted as the percentage OR-I7 activation, thus the measurement is relative to the C_8 activity level.

In a study examining ligand-olfactory receptor specificity of the OR-I7 receptor, 90 odorants were examined, where functional groups, backbone chain length, degree of unsaturation, and side chain substitutions were assessed for their binding properties with OR-I7 (Araneda et al 2000). Although many compounds excited OR-I7 (Araneda et al 2004) between 33 and 55 receptors bind C_8 yielded the greatest level of excitation in I7 (Singer 2000) indicating receptor specificity may be based on potency. A review (Mombaerts 2004) outlined the olfactory pathways and the receptor-ligand. Most mammalian olfactory research has been done on rats and mice; however, there are few studies that have examined the ORI7-40 olfactory receptors in humans, which have been identified as a helional receptor (Levasseur et al 2003, Mombaerts 2004, Spehr et al 2003). The homology of ORI7-40 resembles that of OR-I7 in that rat but is a different receptor. Helional is a citrus smelling synthesized compound available from IFF. This research identified that an increased calcium response (increased activation) was both dependent on the ligand as well as dose.

Based on the original EOG recordings of the mammalian OR-I7 receptor, it is clear that there is little activation of OR-I7 by hexanal (C_6) and an increased activation by C_7-C_{10} and little activation by C_{11} (Zhao et al 1998). In 1990 odor detection thresholds were published for C_6, C_7, C_8, C_9, and C_{10} (Leffingwell & Leffingwell 1991, Nagata & Takeuchi 1990). Upon transformation of the detection thresholds into odor activity values, it is clear that there is a strong relationship between detection threshold and the mammalian olfactory receptor activation by these straight chain aldehydes – C_8 yielding the strongest response (see Figure 1.3). Percent odor activity was calculated using this formula:

$$\%OdorActivity = \left(\sqrt{\frac{X_{threshold}}{C_{8\_threshold}}}\right)*100$$

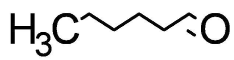
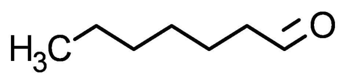
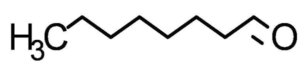
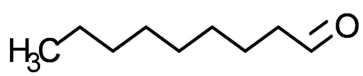
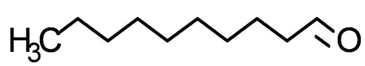
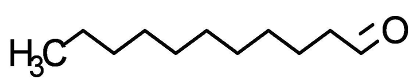
Where X, is defined as the threshold of an odorant. The ratio is taken of the square root to reflect the exponential nature of odorant dose-response behavior. It is important to note that although C_8 demonstrated the greatest excitation when bound to OR-I7, there were small levels of excitation for aldehydes C_6 and C_{11} (undecanal), thus demonstrating the broad molecular range of the olfactory receptors (Araneda et al 2000, Araneda et al 2004).

1.5 Perception

The olfactory experience of detecting an odor is the result of a signal relayed to the brain through olfactory transduction. Psychophysics examines the quantitative relationship between the stimulus and the resulting sensory response. Psychophysics is utilized to determine a quantitative relationship between the stimulus and its psychological impact (Gescheider 1997). Humans are able to quantify experiences such as odorant intensity when given a scale and are able to compare odorant intensities to distinguish differences.

In order for an individual to have an olfactory perception, a person performs two separate psychological processes. The first is the translation of stimulus intensity into a subjective experience. The second is the conversion

Table 1.1: Aldehydes C_6-C_{11}, structure, molecular weight, odor character, and detection threshold. All odorants are reagent grade and have greater than 97% purity.

Compound	Structure	CAS #	Molecular Weight	Odor Character	Detection Threshold
Hexanal (C_6)		66-25-1	100.1	Grass, tallow, fat	0.0045-5[a]; 0.00028[b]
Heptanal (C_7)		111-71-7	114.1	Fat, citrus, rancid	0.003[a]; 0.00018[b]
Octanal (C_8)		124-13-0	128.0	Fat, soap, lemon, green	0.0007[a]; 0.00001[b]
Nonanal (C_9)		124-19-6	142.1	Fat, citrus, green	0.001[a]; 0.00034[b]
Decanal (C_{10})		112-31-2	156.2	Soap, orange peel, tallow	0.0001-2[a]; 0.00040[b]
Undecanal (C_{11})		170-29	170.29	Oil, pungent, sweet	0.005[a]

* Published percepts listed on the Flavornet (Arn and Acree, 1998). [a]published detection thresholds ppm in water listed in Leffingwell et. al.[b] thresholds in ppm v/v listed in Nagata (1990).

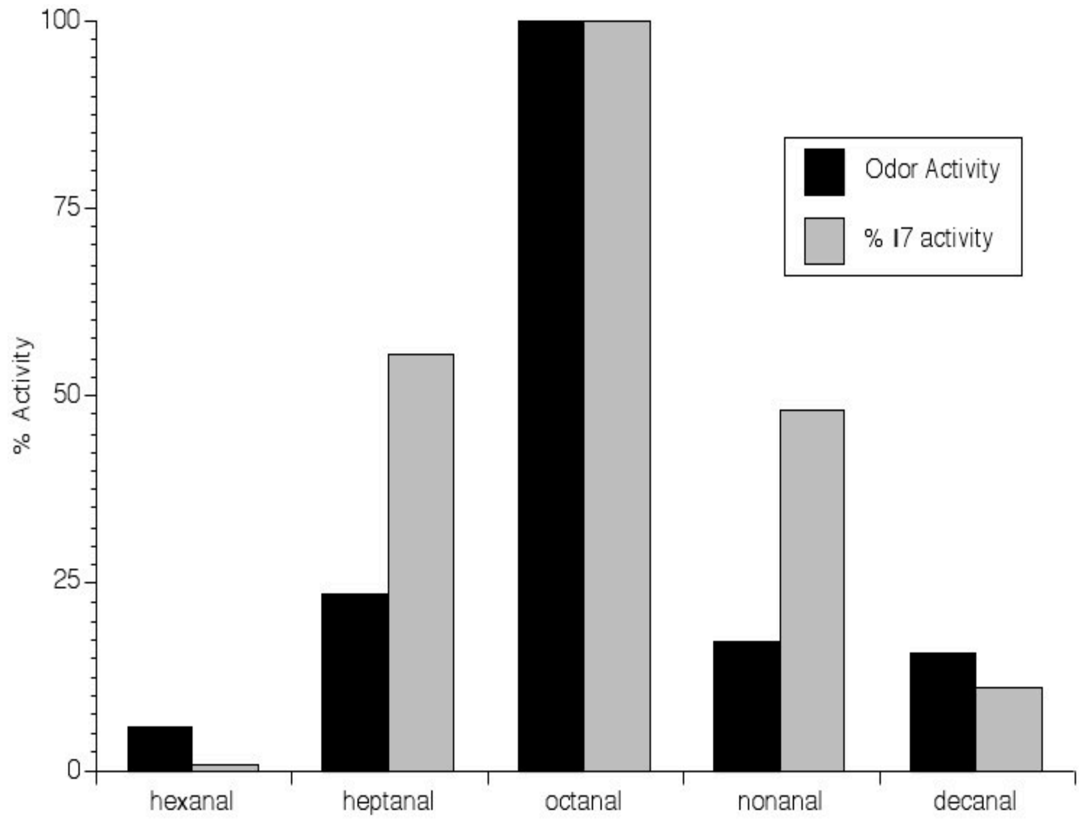


Figure 1.3: Nagata (1990) odor activity and Zhao et. al. (1998) %I7 activity data compared. Both the physiological and psychometric data indicate C_8 is the primary agonist.

of the perception into a measurement, such as the perception of numbers, line lengths, force or other response categories (Lawless & Heymann 1998). Sensory psychology is the study of the psychophysical process involved in the translation of the stimulus intensity while sensory testing measures these perceptions experimentally.

The sensory threshold is central to the concept of psychophysics. One of the earliest definitions of threshold written by the philosopher, Herbert (1924) stated that in order to experience a mental event the stimulus had to be stronger than a critical amount. In the early nineteenth century scientists, E.H. Weber and G.T. Fechner determined methods to measure the sensitivity limits of humans and created a new definition for absolute threshold or stimulus threshold. They defined this as the stimulus level below which no sensory response could be detected, and above which the detectable response increased in intensity with ever-greater stimulus response levels until a maximum response was achieved (Gescheider 1997). When a stimulus is above the absolute threshold stimulus intensity can be increased or decreased to produce a just noticeable difference.

A dose response curve is often used to describe both the behavior of taste and smell and is best characterized by the sigmoidal cumulative distribution function (CDF) – the integral of Gaussian and binomial distributions. There is no single point of discontinuity on the CDF that corresponds to the original threshold definition, thus there are numerous threshold definitions all of which reside within a “threshold zone.” The threshold definition most commonly used is based upon the midpoint of a psychophysical function. The psychometric function is constructed from the probability of stimulus detection, rather than from the quantification of perceived intensity (Marin et al 1991). There is large variability in the thresholds of a population compared to the threshold of an individual.

This variability is the result of individual differences (Keller et al 2007, Menashe et al 2003) individual variation, and in part to poor measurement technique. Individuals with reportedly normal olfactory response may display a decreased response to a particular odorant; this phenomenon would be defined as a specific anosmia or an odor-specific sensory deficit (OSD). OSDs can be detected using threshold measurements of pure stimulants and subjects screened for the presence of specific anosmias. In the research reported, thresholds were used to determine detectible range of odorants concentrations for the subjects.

1.6 ADAPTATION

Adaptation is defined as a reduction in sensitivity to a particular stimulus due to prolonged exposure. Physiological adaptation research by Kurahashi and Menini (1997) determined the mechanism driving odorant adaptation is the modulation of the cAMP-gated channel regulated by Ca^{2+} feedback. A second messenger, cAMP, is required for the transduction of an olfactory signal. cAMP activates the opening of Ca^{2+} activated chloride channels. The signal is trandsduced and sent for higher cortical processing. In cells, adaptation can be measured as a function of electronic current activity caused by differences in ion potential from the opening and closing of Ca^{2+} gated channels. Upon initial exposure to an odorant the cells shows an initial rise in activity due to the immediate detection of the odorant, and a subsequent decrease in activity as it reaches a plateau (Firestein & Shepherd 1990, Firestein et al 1990, Kurahashi & Shibuya 1990, Torre et al 1995). Ca^{2+}

gated channels are required for the feedback regulation in cells and are considered to be responsible for the reduced excitation during prolonged exposure leading to adaptation. Several studies have examined mitral and tufted cells in the anterior piriform cortex (aPCX) and discovered a short-term depression in activity after continuous exposure to an odorant. This synaptic depression represents both adaptations at the neural level as well as result in behavioral adaptation (Best & Wilson 2004, Wilson 1998, Yadon & Wilson 2005). Thus odorants sharing a common OR are more likely to cause adaptation to one another than odorants that do not share a common receptor site.

In psychophysics adaptation is defined as an increase in detection threshold of an odorant due to repeated exposure. Thus sensitivity to the exposed odorant decreases. There are two different types of olfactory adaptation: self-adaptation and cross adaptation. Self-adaptation is a decrease in sensitivity to an odorant when exposed to the same odorant for a prolonged period of time. Cross adaptation is a decrease in olfactory sensitivity to a particular odorant due to exposure to a different odorant. Examining odorants which cross-adapt can reveal new properties shared by an odorant such as a common receptor site. A common example of olfactory self-adaptation occurs when an individual enters a smelly room, after several minutes the off-odor disappears. However, upon leaving and re-entering the room, the subject will again detect odor in the room. During adaptation sensitivity is temporarily decreased and recovers once exposure to the odorant has ceased.

1.6.1 FACTORS EFFECTING ADAPTATION

A review (Dalton 2000) described the several different factors influencing olfactory adaptation. One factor influencing one's ability to adapt to an odorant is dependent upon the odorant being tested. Individuals are able to adapt to some odorants instantaneously, while other odorants take much longer. Vaschide (1901) conducted one of the earliest studies demonstrating this phenomenon using ether, ammonia, and camphor. He found that adaptation was faster for both ether and ammonia while after over 30 minutes of exposure to camphor; camphor could still be perceived (Vaschide 1901). Some odorants, particularly pungent odors, as well as odorants stimulating the trigeminal nerve, are less sensitive to adaptation (Dalton 2000).

Another factor influencing olfactory adaptation is the intensity of the adapting stimulus. In 1920, Zwaardemaker, conducted several studies examining the relationship between the intensity of the odorant and the time necessary for the onset of adaptation. He found, as the intensity of the odor increased, the detection threshold increased, thus a stronger intensity of the odorant was required in order for detection (Zwaardemaker 1895, Zwaardemaker 1920). Furthermore, as the duration of exposure increased, the intensity of the odorant had to be increased for further detection of the odorant. However, as previously mentioned the effect of the intensity of time of exposure is dependent upon the odorant to which the subject has been exposed (Wuttke & Tompkins 2000). The length of exposure additionally affects the rate of recovery from adaptation (Berglund et al 1971, Berglund et al 1976, Cain 1974).

Odorant intensity is crucial in determining the effect of adaptation upon an organism. Stone et. al., 1972, examined the effects of varying odor concentrations of ethyl acetate and proponoic acid on drosphilia larvae. As the concentrations of the two odorants increased, the degree of adaptation increased. Further exposure to the odorants yielded a decreased response.

As mentioned earlier, the time necessary for an individual to recover from the effects of adaptation is dependent upon the odorant of exposure. In a study conducted by Aronsohn in 1886, subjects were exposed to coumarin and eu de cologne. Aronsohn noted that it took 120 seconds to fully adapt to coumarin and 65 seconds to fully adapt to the eau de cologne; however, it took over three minutes for subjects to fully regain sensitivity to these odorants. Further studies examining the recovery time after adaptation by Elsberg (1935), Koster (1965), Stuiver (1958), Dalton (1996), and Wysocki (1996) have all shown that plotting the recovery curves from adaptation is dependent upon the individual and the odors to which he or she were exposed (Dalton & Wysocki 1996, Elsberg et al 1935, Koster 1965, Koster 1968, Koster 1971, Stuiver 1958). The shape of the recovery curve is often irregular; however, there is one common fact, which underlies olfactory recover: adaptation is a must faster process than recovery.

1.6.2 *CROSS-ADAPTATION*

Several studies have demonstrated decreases in sensitivity to an odorant resulting from exposure to a different odorant. Increases in detection threshold due to exposure to a different odorant are caused by two different mechanisms, similar odor quality or similar chemical functionality (Pierce et al 1996). Studies of cross-adaptation date back to 1886, and have examined the suppressive effects of one odor's effect on the ability to detect a separate odor (Arohnsohn 1886, Backman 1917a, Backman 1917b, Backman 1917c, Cain &

Polak 1992, Cheesman & Mayne 1953, Engen 1962, Engen 1964a, Engen 1964b, Gottfried et al 2006, Hermanides 1909, Koster 1971, Laska & Teubner 1999, Nagel 1904, Parker 1922, Todrank et al 1991, Vaschide 1901). The overall conclusion of these studies is that not all odorants can cause cross-adaptation effects; however, some odors can cause a decrease in sensitivity for another odor.

E.P. Koster (1971) performed several studies examining the cross-adaptation of several different substances and determined several factors influencing the cross-adaptation of odorants (Koster 1971). First, sensitivity to a substance different from the adapting stimulus cannot be enhanced. Secondly, a substance cannot reduce the sensitivity to an odorous substance more than the substance itself, thus self-adaptation will always be stronger (display a greater reduction in sensitivity) than cross-adaptation. Furthermore, cross-adaptation is not always symmetrical. An example of asymmetrical cross adaptation would be if an individual were adapted to a floral odor by smelling an adapting citrus odor stimulus, but does not adapt to the citrus odor when the adapting stimulus is floral. Thus it is possible adapt to a floral odor and become desensitized to a citrus odor, then subsequently expose oneself to citrus odor and be highly sensitive to the floral odor. Additionally, some odors are more easily cross-adapted than others. Substances with similar odor profiles may not always cross-adapt (Koster 1971). Although C_6 is characteristically different than C_8 it is possible that these odorants may cross-adapt; however, it would be more likely that odorants C_8 and C_{10} would cross-adapt due to their common activation of OR-I7.

More recent studies continue to support E.P. Koster's findings, a study conducted by Cain and Polak (1992) examined two substances with similar bitter chocolate percepts. In this study trimethyl pyrazine (TMP) and 2-Propionyl-3-Methyl Furan (PMF) were examined for cross adaptability (Cain & Polak 1992). Both TMP and PMF exhibit a similar odor quality; three other compounds with different odor quality and but similar chemical functionality were examined. PMF and TMP share a common odor but different chemical structure; however, both odorants cross adapt with one another. Subjects exposed to the three odorants with similar structure but different odor quality did not display decreased sensitivity.

In a separate study conducted by Pierce et. al. (1996) two structurally similar odorants were analyzed the odor quality of the two odorants were extremely different; however, they shared a common chemical functionality group and display cross-adaptation (Pierce et al 1996). Isomeric mixtures of (E) and (Z)-3-methyl-2-hexenoic acid, the (E) isomer is the primary odorant in underarm sweat and an isomeric mixture of ethyl esters (E) and (Z) 3-methyl-2-hexonoic acid, a fruity odor. The ethyl esters of organic acids are structurally similar to the corresponding acids, the acidic hydrogen is replaced by CH_2 CH_3 (ethyl) moiety (Pierce et. al. 1995). The odorant characterized as underarm sweat is extremely similar in structure to an odorant known to be fruity, although these odorants are extremely perceptually different these odorants asymmetrically cross-adapt. Thus the order of presentation effects the reduction in sensitivity.

1.6.3 *CROSS-ADAPTATION EXAMINED THROUGH fMRI*

Cross-adaptation of odorants has also been analyzed using fMRI. In a study conducted by Gottfried et. al. 2006, odorants with similar structure as well as odorants sharing a similar odor quality were analyzed for their excitation of the piriform cortex. This study found that structure and odor quality are most likely processed separately. Further findings demonstrated that as odor quality similarity increased, blood flow to that particular region in the piriform cortex decreased indicative of adaptation. Odorants with similar chemical structure and non-similar odor did not display adaptation effects (Gottfried et al 2006).

1.6.4 *CROSS-ADAPTATION IN OR-I7*

In relation to the OR-I7, receptor where C_8 has been identified as the primary agonist, odorants C_7-C_{10} have been identified to excite the I-7 receptor as well. In 2006, Kittel conducted a free-choice profiling of odorants C_6-C_{12}. Odorants C_7-C_{12} were identified as exhibiting citrus qualities ranging from rancid, oily, and nutty to fruit, soap, and floral. Unlike the other substances, C_6 did not fall into these categories. Instead subjects separately categorized C_6 as musty, grass, and green (Kittel et al 2008). As mentioned earlier there is little electrical activity elicited when OR-I7 is exposed to C_6, indicating that OR-I7 is more responsive to aldehydes with citrus qualities (Kittel et al 2008). Both C_{10} and C_{11} have pungent qualities that elicit activity from the trigeminal nerve. Cross-adaptation would be expected between similar odorants C_8 and C_9 as well as C_9 and C_{10}. Based on the physiological rat recordings and FCP data it can be predicted that C_7 and C_8, C_8 and C_9, C_9 and C_{10}, and C_{10} and C_{11} would display evidence of cross-adaptation (Laska & Teubner 1999). However, it would be expected that cross-adaptation be extremely low between C_6 and C_7, C_8, C_9, C_{10}, C_{11}. Odorants that do not cross-adapt most likely do not share a common receptor site although this has yet to be proven.

1.7 MIXTURES

1.7.1 *Mixture Suppression*

Odor mixture perception is not well understood although there are several principles that are common to mixtures. These mixture principles are illustrated in figure 1.4. The intensities of individual component odors are stronger than the intensities of the component odors in a mixture. This phenomenon is known as odor mixture suppression and it has been reported both in smell and in taste (Bartoshuk 1975, Cain & Drexler 1974, Lawless 1987). Jones and Woskow (1964) were the first to determine that the intensities of individual odorants are not equal to the overall intensity of the mixture (Cain & Drexler 1974, Jones & Woskow 1964). The intensity of an individual odorant is strongest alone, the addition of other odorants results in suppression as illustrated in figure 1.4. Complete addition occurs when the perceived magnitude of individual odorants is the same as the perceived magnitude of the odorants when unmixed. There have been many studies supporting the phenomenon of mixture suppression where the results clearly demonstrate hypoadditivity (Bell et al 1987, Berglund & Olsson 1993a, Berglund & Olsson 1993b, Derby et al 1996, Derby et al 1984, Laing et al 1984, Laing & Wilcox 1983). Although the intensity of a mixture is always less than the absolute addition of the intensities it's components. It is understood that the intensity of a mixture is never less than the intensity of the weakest component (Laing & Wilcox 1983). It is unclear why mixture suppression reportedly occurs in almost all mixtures, even in odorants that are known to not cross-adapt. One possibility is that mixture suppression is a process separate from odor adaptation.

1.7.2 *ODORANT SIMILARITY AND DISSIMILARITY*

Several studies have examined the number of components one is capable of detecting in a mixture. Laing and Francis (1989) determined that within a complex mixture, there is a limited capacity for the identification of components. Individuals are capable of identifying three odorants successfully in a complex mixture of three odorants. Mixtures of four and five components resulted in large numbers of incorrect odor judgments (Laing & Francis 1989, Laska & Hudson 1993). Further studies have yielded similar results. In another study conducted by Livermore and Laing (1998) subjects were asked to discriminate odors within a mixture containing up to eight different components comprising the mixture. This study examines the concept of good blenders and poor blenders (Livermore & Laing 1998). It would be expected that poor blenders would be easier to discriminate; however, regardless of odorant type the maximum number of components capable of being successfully identified within a mixture was four (Livermore & Laing 1998). For the OR-I7 odorants C_8 and C_{10} would be considered good blenders, while C_6 and C_8 would be poor-blenders due to their difference in odor quality. Although odor type can skew the ability to detect odorants within a mixture the limited capacity of odorant identification within a mixture is independent of odor type. The identification of an upper limit for odor identification within mixtures has been further confirmed by the findings of Livermore & Laing (1996) and Goyert et. al. (2007) (Goyert et al 2007, Livermore & Laing 1996).

1.7.3 *MIXTURE PROCESSING*

There are two established theories of mixtures perception, elemental and configural (combinatorial). The elemental theory of odor mixture

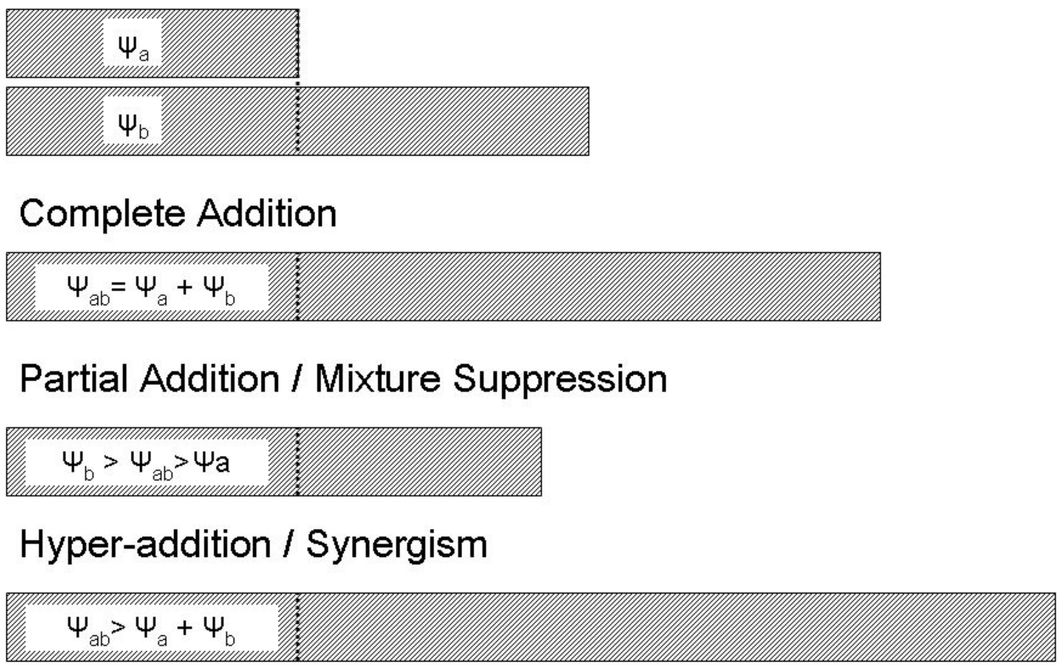


Figure 1.4. Adapted from Cain and Drexler (1974). The dashed line marks the intensity of the weakest component. Complete addition is when intensity of the mixture is equal to the intensity of the components. Mixture suppression occurs when the total intensity of the mixture is less than that of the individual components. Synergism occurs when the total intensity of the mixture is greater than the intensities of the individual components. The intensity of a mixture is never less than the intensity of the weakest component.

perception states that mixtures are the sum of their components, each individual odorant can be detected within the mixture, and no novel odors are created through the mixture process. The configural theory of odor mixture perception states that mixtures create a novel odorant an emergent property is created through the combining of individual odorants (Kay et al 2003). As previously discussed there is a defined upper limit for detecting individual components within mixtures; the configural theory would argue that these limits exist due to the fact that odorants are changing within the mixture. It must be noted that mixtures do have a unique mixture odor that is different than the ability to detect odors within the mixture. Le Berre et. al. (2008) examined the role of component odors in creating a complex odor mixture perception. Component odors were combined to create the perceptions of pineapple and grenadine. Individually panelists did not perceive the component odors to be characteristic of their complex mixtures; however, when combined the components were reflective of the mixture. Thus the mixture smell is the result of component odors. The difference between a mixture odor and the two processing schemes is that according to configural processing the individual components would not be detectable within the complex mixture.

1.7.4 *ELEMENTAL THEORY*

The elemental theory of mixture perception states that all components are present and detectable within a mixture; however, detection may be reduced due to other components causing mixture suppression. Additionally, the different components within a mixture may become clearer over time due to adaptation to the dominant odorant within the mixture. The first studies of elemental processing were conducted on the simplest mixture—binary mixtures. These studies examined similar and dissimilar odors. Similar odors are hypothesized to be more difficult to detect in mixtures than dissimilar odors. Laing et. al. (1984) observed that when mixtures were of similar odor intensity, the components were able to be perceived (Laing et al 1984). However, when the components were of unequal intensity within the mixtures suppression occurred, where a strong odorant suppressed the intensity of the weaker odorants present within the mixture (Cometto-Muniz et al 2005, Cometto-Muniz et al 1999, Laing & Wilcox 1983)

1.7.5 *THE INFLUENCE OF ODOR QUALITY ON ADAPTATION*

Furthermore, the overall odor quality of the mixture is dependent on the intensities of the components present within the mixture. Quality is defined as the odor type and whether the odorant may activate the same receptor or different receptors. Additionally the quality of the mixture changes depending on the ratio of the intensities of the components within the mixture (Laing & Wilcox 1983). In a study examining the influence of odor type on discrimination and identification of odorants within complex mixtures Livermore and Laing (1998) determined that although dissimilar odors facilitated the identification of odorants within a mixture, the number of components within the mixture ultimately influenced the identification process (Livermore & Laing 1998). This area of dissimilar and similar odors has been researched in depth, demonstrating that the perceptual similarity between two odors predicts the mixture interaction properties of the odorants presented within the mixture (Derby et al 1996, Jinks & Laing 1999, Kay et al 2003, Laing & Francis 1989, Laing et al 1984, Laing & Willcox 1987, Laska & Hudson 1993, Linster &

Cleland 2004, Linster & Smith 1999, Smith 1996, Staubli et al 1987, Wiltrout et al 2003).

When mixtures consisting of perceptually similar odorants are presented, the mixture may appear as a novel percept (configural), whereas when presented with dissimilar odors the dissimilar odors persist within the mixture. Laing and Wilcox (1983) found that a new odor was not formed as a result of the blending of dissimilar odorants (Laing & Wilcox 1983). Research has also shown that familiarity and pleasantness may also influence the ability of individuals to detect odors within a mixture (Laing & Francis 1989, Rabin & Cain 1984, Staubli et al 1987).

1.7.6 *TEMPORAL*

There is also a temporal aspect to elemental processing, where depending upon the time of presentation between odorants in a mixture; individuals can distinguish odorants (Jinks & Laing 1999, Laing et al 1994). In a study conducted by Jinks and Laing (1999) subjects were incapable of identifying four different components within a mixture when presented simultaneously. However, when presented with three components over a period of time, thus creating a mixture by adding odorants over a period of several seconds, subjects were able to identify the three components (Jinks & Laing 1999). Jinks and Laing (1999) concluded that the difficulty in temporal processing of odor mixtures might be the result of limitations to the olfactory processing working memory. The presentation of more than three odorants simultaneously may overload this memory bank.

1.7.7 *CONFIGURAL (COMBINATORIAL) PROCESSING*

As mentioned previously ORs bind a range of ligands. Olfactory information converges at the glomerulus.

The ability of cortical neurons to integrate information in the olfactory cortex creates the possibility for new novel percepts to be created. The information combined in the olfactory cortex may explain the difference between the perception of mixture components versus the mixture. Zou and Buck (2006) demonstrated that binary odorant mixtures were capable of stimulating cortical neurons which were not stimulated by individual components. Thus olfactory information from mixtures creates novel combinations of receptor inputs which are not stimulated by the single odorants (Zou & Buck 2006). There is still not enough information confirming the validity of either the configural or elemental olfactory process; however, there have been some interesting observations made concerning the ability to detect odorants within mixture.

1.7.8 *SIMILAR AND DISSIMILAR ODORS IN MIXTURE PROCESSING*

Several studies found that mixtures using odorants with similar qualities were much more difficult to distinguish than mixtures composed of dissimilar odorants. Laing and Francis (1989) noted that the difficulty their subjects had in identifying odorants within complex odor systems might have been due to blending. Training has been shown to greatly increase the ability to identify components within a mixture. Several studies have shown that after training, subjects were able to identify components within mixtures, which were previously unidentifiable (Kay et al 2003, Livermore & Laing 1996, Mandairon et al 2006, Wiltrout et al 2003).

In a study conducted by Mandairon et. al., (2006), rats were exposed to several different odor mixtures. When the rats were initially exposed to the mixtures prior to a 20-day enrichment period their actions indicated that the mixtures were configural (synthetic) and were unable to identify the components within the present mixture. However, after the 20 day enrichment period whereupon the rats were continuously exposed to the components within the mixtures the rats were capable of identifying components within the mixture. Even more telling, the rats were able to discriminate between odorants that they were not trained to discriminate against (Mandairon et al 2006). Odorants that appear as similar to an untrained individual may have slight but distinguishing differences that would not be apparent to the untrained individual – thus training is essential to examine odorant mixture perception individuals (Harper et al 1967). Mixtures that might appear to be novel may only be novel to the untrained nose. These studies demonstrate the importance of prior-exposure and training for the evaluation of odorants within mixtures, thus training is an integral part for understanding odor mixture perception.

1.7.9 *RELEASE FROM SUPPRESSION*

As previously mentioned, odor mixtures display mixture suppression. However, if an individual is adapted to one of the components within a mixture and then smells the mixture again the other components within the mixture will appear as stronger. This effect is known as release from suppression, where the components within the mixture that have not been adapted appear as stronger in intensity. In a study conducted where subjects were presented with two stimuli cinnamaldehyde and vanillin, when subjects were adapted to cinnamaldehyde and subsequently presented with a mixture of cinnamaldehyde and vanillin, subjects rated the vanillin as the stronger component within the mixture (Lawless 1987). Furthermore, when subjects were adapted to vanillin and subsequently exposed to a binary mixture of cinnamaldehyde and vanillin, subjects rated the cinnamaldehyde as the stronger stimulus within the mixture (Lawless 1987).

Thus upon adaptation to one component in a mixture, when presented with the mixture the other components appear stronger creating a contrast effect. Contrast effects occur when a target stimulus is perceived as more extreme when in the presence of another stimulus than the target stimulus would have been in isolation (Lawless & Heymann 1998). In olfaction, contrast effects often occur in the presence of a background stimulus, often causing a particular odor to “pop out” from the mixture.

Although according to the configural theory component odors are synthesized into novel odors, individuals are still capable of detecting odorants even with the presence of a constant background (Dalton 2000, Goyert et al 2007, Lawless 1987, Stevenson 2001, Stevenson et al 2007). In a series of experiments where subjects were adapted to a mixture of N components, (N varied from one to three components), subjects were presented with a test stimulus N+1. Subjects were asked to identify the +1 component, which was not present within the adaptation stimulus, therefore based on the principles of release from suppression the newly added odorant should be identifiable. This technique of selective adaptation allowed subjects to successfully identify the new additional component within the mixture due to selective adaptation (Goyert et al 2007). Thus, odor identification is possible within in a four-component mixture or perhaps even more complex mixtures, by using selective adaptation as well as training techniques.

1.8 FIGURE-GROUND PROCESSING IN VISION

Edgar Rubin first defined figure-ground relationships in 1915. Rubin created the Rubin vase, one of the most recognizable images in figure-ground gestalt (Figure 1.5). The Rubin vase is formed by the profiles of two faces, which create the image of a vase. Depending on which image the viewer defines as the figure, the viewer either sees the vase or the two faces but normally does not see both images simultaneously. According to Rubin "What is perceived as figure and what is perceived as ground do not shape in the same way. In a certain sense, the ground has no shape" (Yantis 2001). Several observations have been made concerning figure-ground principles in vision. The first is that the figure is more "thing like" and more recognizable than the background (Goldstein 2001). Secondly, the figure is perceived as in front of the ground. Thirdly, the ground is perceived as unformed and extends behind the figure. Lastly, the contours separating the figure from the ground appear to belong to the figure (Goldstein 2001). Several more observations concerning figure-ground visual images have found that stimuli with smaller areas are more often seen as figure (Kunnupas 1957, Oyama 1960). Additionally, vertical and horizontal lines are more likely to be perceived as figure than are other line orientations. Finally meaningful objects are more likely to be seen as figure (Goldstein 2001). One theory for viewing objects one at a time, such as in Rubin's vase, is that it is extremely unlikely that there would be two faces and a vase that would share the same contours, the visual system thus assumes the most likely occurrence that the contour separating the two regions belong to one object – hence the area that belongs to the contour becomes the figure and the other is identified as the ground (Baylis & Driver 2001).

There are several competing theories concerning identifying the meaningfulness of objects within a scene. One theory suggests that the figure is segregated from the ground followed by recognizing the meaningfulness of the figure. (Peterson 1994), using a black and white image where half of the picture appeared as a silhouette of a woman, identified that subjects recognized the woman as the figure in the image. However, when Peterson flipped the picture upside-down, making the identification of the silhouette more difficult,
subjects were less
likely to identify the black silhouette as the figure in the image. This is evidence suggests that segregation does not always precede recognition (Vecera & O'Reilly 2000). Baylis and Driver (2001) have demonstrated in the macaque that neurons in the inferior temporal cortex (IT) respond to visual shape and are derived after the assignment of figure-ground contours (Baylis & Driver 2001).

Visual assignment is a key part of feature integration theory (FIT), where object perception is theorized to occur in a series of stages (Treisman, 1987, 1993, 1998). In the preattentive stage the visual system examines and image, and determines the existence of the features that comprise the basic units of perception: curvature, orientation, ends of lines, color, and movement. In the focused attention stage, features are combined to create and object. In order to determine the basic features of an image “pop-out” boundaries are determined where areas are composed of different elements

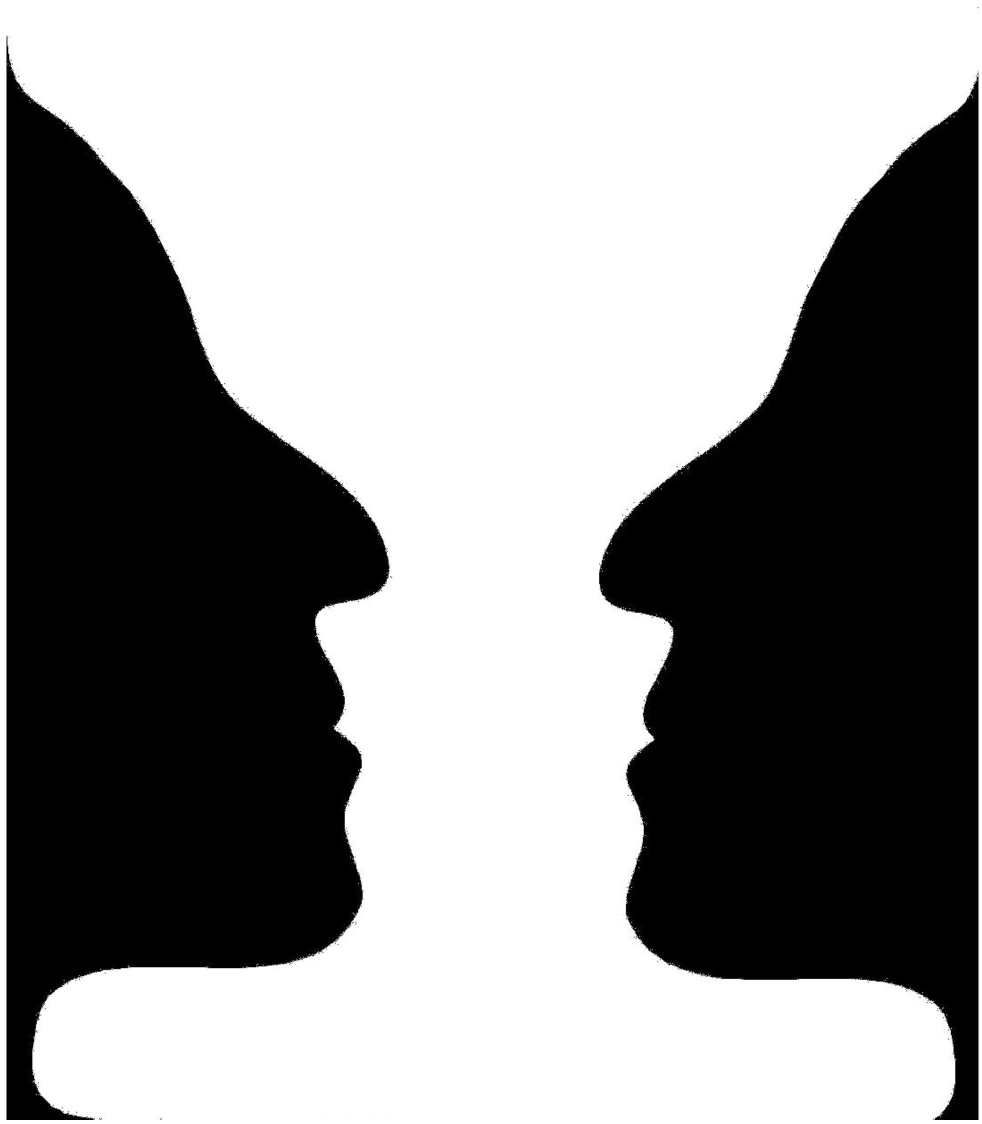


Figure 1.5: Rubin's vase. The viewer either sees two faces in profile, or a vase. The viewer often does not see both images simultaneously.

(Goldstein 2001, Julesz 1981, Kandel et al 2000, Treisman & Gormican 1988). Pop-out boundaries are determined when two sets of elements are displayed next to each other, if the two areas contain different features or different values of the same feature, then the boundaries pop out between the two areas. In a visual search subjects are presented with a display where several elements are presented, the subject is instructed to find one particular element (Treisman & Gormican 1988). When a target "pops-out" search time is much shorter. Curvature, tilt, line ends, movement, color, brightness, and direction of illumination, are basic features which lead to "pop-out" effects in visual search tasks (Beck et al 1989, Julesz 1984, Treisman & Gormican 1988). These features are determined during the pre-attentive stage.

In the focused attention stage the various features that compose the objects must be combined before an individual perceives an object. The image is decomposed into features, with attention the features within a particular location are combined whereupon they are compared to memory (Treisman, 1993). Treisman's theories support the idea of feature integration, where there is early feature extraction and processing. A separate theory of object recognition is recognition by components (RBC) (Biederman 1987) Biederman determined that there are features, geons—volumetric primitive objects. According to Biederman and object or scene is analyzed into these geon (cylinders, rectangular solids, pyramids) parts and can be constructed into thousands of objects. Figure 1.6 diagrams the cognitive processes used to separate figure from ground.

Another theory examines neural feature detectors and approaches object perception through physiology. In several different neurological studies Hubel and Wiesel (1963) (Wiesel & Hubel 1963) identified neurons with specific functions for orientation. They identified that there are specific columnar cells on the visual cortex that respond to different orientations, as well as cells which respond best at 45 degrees and cells which respond best at a 40 degree angle. The identification of columns of cells in the cortex excited by particular orientations shows the neurological aspect of vision and contour identification (Hubel & Wiesel 1959, Hubel & Wiesel 1962). Visual feature identification occurs due to several different attention processes as well as several different types of cells in the visual cortex being excited to create and image which we can refer to in our memories to identify the meaningful objects.

1.9 FIGURE-GROUND PROCESSING IN OLFACTION

Olfaction, like vision, is a very complex system. In order to identify components of a mixture there must be a separation of the figure (odorant X) from the ground (ambient mixture). Like in vision, there are several different theories, which examine whether information is combined to create an image or information is used to deconstruct an image. The ability to distinguish a gas leak through the detection of mercaptans, from the common smell of one's household is one way in which individuals experience figure-ground distinctions in everyday life. There are also several neurological studies examining where in the cortex olfactory mixture processing occurs. Recently the anterior piriform cortex (aPCX) has been identified as an area in the brain responsible for figure-ground separation in the olfactory cortex (Best & Wilson 2004, Gottfried et al 2006, Kadohisa & Wilson 2006).

The work by Best and Wilson (2004) demonstrated that upon exposure and adaptation to one odor, the aPCX neurons display synaptic depression.

Furthermore, when exposed to a new odor these neurons are unaffected showing no cross adaptation (Best & Wilson 2004). Therefore, once the cortex has been adapted to one odor and the odorant remains present in the background the introduction of a new second odorant, is treated as a new odorant in isolation from the background. Thus aPCX neurons contribute to figure-ground separation. According to Kadohisa and Wilson (2005) aPCX neurons may allow for figure-ground separation, aPCX neurons filter out the background stimulus from the newly introduced odor (Figure 7). In this study when aPCX neurons were exposed continuously to a single odorant, A, over a period of 40 seconds there was a reduction in the neuronal activity of the cell. However, when delivering a continuous stream of air containing substance and introducing a new odorant, B, for a few short seconds, there was a sudden spike in aPCX neuronal activity (Kadohisa & Wilson 2006). Suggesting that aPCX neurons are capable of filtering background odors from newly introduced odors, thereby creating a figure-ground relationship (Kadohisa & Wilson 2006). Mitral and tufted cells located in the olfactory bulb are unable to display this excitation relationship; instead they continue to respond to the background odorant instead of filter the information.

The possibility that aPCX neurons are capable of filtering olfactory information to create figure-ground relationships further supports the elemental theory of processing. When perceiving mixtures there is usually an odorant which is perceived as dominant, where all other odorants within the mixture are perceived as background odorants although still present within the mixture.

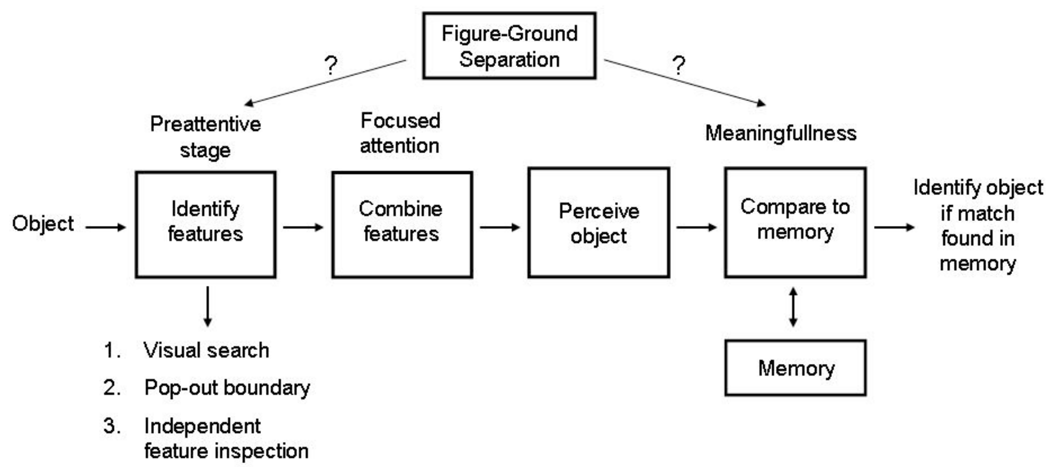


Figure 1.6: Flow diagram of Feature Integration Theory (FIT) developed by Triesman. Where figure-ground separation occurs is debated in the literature. Modified from Goldstein (2001).

1.10 METHODS OF ANALYSIS FOR THIS STUDY

1.10.1 *METHODS OF PRESENTATION*

Odorants are commonly presented in 250 mL polyurethane squeeze bottles, where perfume blotters have been dipped in the odorant solution and placed in the bottle to equilibrate (Goyert et al 2007).

In order to properly examine odor detection it is necessary to determine that the concentrations of the odorant stimuli to be examined are within the detection range for the subjects. Several different methods have been devised for determining detection levels of odorants. The most commonly used method for determining concentration-detection (psychometric) functions for odors of a single chemical is to use a 3-alternative, forced choice (3-AFC) procedure, where odorants were presented in order of ascending concentration. Subjects are presented with three bottles, subjects are asked to identify the bottle containing the odorant (Cometto-Muniz et al 2005). Subjects are asked to indicate which bottle contains the odor, and even guess if unsure (hence the forced choice). On each trial two of the bottles contain solvent, and the third bottle contains the odorant at a particular concentration. The position of the blank is randomized. In a test series, subjects evaluate odorants of each dilution step at least twice, in ascending order of concentration. Between presentations of each trio there is commonly a 30-45 second break. Each bottle is squeezed/ sniffed once, which is enough to determine whether the odorant is present (Laing 1986). Upon identification of the bottle containing the odorant subjects are asked to evaluate the intensity of the odorant within the bottle on a 9-point intensity scale where 1= no odor present, 9= very strong. In other methods subjects might be asked to judge rate their confidence in their judgment on a 1-5 confidence scale, where 1= not confident; 5= extremely confident (Cain & Schmidt 2002, Cometto-Muniz et al 2005).

1.10.2 *ADAPTATION METHODS*

In order to evaluate changes in odorant intensity resulting from adaptation, it necessary to evaluate the perceived intensity of the odorant prior to and after exposure to the adapting stimulus (Cain 1971, Cain & Engen 1969, Colbert & Bergmann 1995, Dalton 2000). Adapting techniques often involve the subject being exposed to an adapting stimulus for several seconds and subsequently making a judgment of the intensity of another odorant. A method adapted from Lawless (1987) involves placing three squeeze bottles containing odorants in front of a subject. The first and third squeeze bottles contain the same odorant (Lawless 1987). The second (middle) squeeze bottle contains the adapting odorant. Subjects are asked to smell and rate the intensity of the bottles from left to right. The subject is asked to squeeze and smell the first bottle and make a judgment of the odorant intensity. The subject is then asked to take 5 deep breathes of the second bottle, and before inhaling asked to squeeze, smell, and judge the intensity of the third bottle. The two judgments allow one to see the change between each trio evaluation there is a break in order for the subject to recover from the effects of adaptation.

1.10.3 *ABX TESTS*

A common method for testing discrimination of samples is the AB-X test as well as sorting task. AB-X tests, asks subjects to match samples to a reference (Huang & Lawless 1998, Lawless & Heymann 1998, Macmillan & Creelman 2005). In an AB-X test, subjects are presented with references A and B and asked to match X to either A or B, this test is a forced choice method for discrimination and has been used in food evaluation as well as audition (Macmillan et al 1977, Pierce & Gilbert 1958). It does identify the difference between the samples, but allows subjects to identify a characteristic that he or she finds common to the references. Sorting tasks have been used as a rapid way to test subject's ability to inspect samples and create categories based on his or her own inspection criteria. In order for sorting tasks to be successful it is necessary that the stimuli evaluated have moderate differences in order to form proper groupings. Sorting has been most commonly analyzed by using multidimensional analysis and thus identifies attributes defining the possible groupings (Heymann 1994, Lawless 1989, Lawless et al 1995). Sorting has been used in order to determine fragrance groupings (Lawless 1989).

1.11 CONCLUSIONS

Odor mixture perception has been reported in research as either an elemental process or configural process depending on similarity and dissimilarity of the odorant properties as well as training. Adaptation studies examining structural and perceptual similarity of odorants have allowed researchers to study the olfactory receptor binding properties of certain ligands. From the results of cross-adaptation studies it can be concluded that odor similarity is more likely to cause cross-adaptation than chemical structure similarity. Knowing that straight chain aldehydes C_6-C_{11} vary in perceptual qualities as well as their binding properties with OR-I7 allows researchers to further probe how the addition of a single carbon to the backbone of an aldehyde changes which olfactory receptors the odorant binds with as well as the perception of the odorant.

Understanding which compounds excite a specific olfactory receptor further our ability to study the perception of odor mixtures. Knowing that C_7, C_8, C_9, and C_{10} favorably bind to OR-I7 while C_6 and C_{11} creates many different research questions, concerning how these compounds cross-adapt and are perceived in mixtures. Adaptation studies can be used to predict the cross-adaptation of these compounds, where it would be expected that C_6 would not cross adapt with C_7, C_8, C_9, and C_{10} due to their different perceptual characteristics as well as their binding with OR-I7. Furthermore, the odor mixture perception of these odorants can be studied as well by combining odorants known to excite the same receptor (C_8 and C_{10}) and odorants that do not excite the same receptor (C_6 and C_8). The objectives of this thesis are to use the tools of psychophysics and sensory evaluation to understand how specific conditions and compounds influence odor mixture processing by examining cross-adaptation characteristics and odor mixture perception.

REFERENCES

Araneda R-C, Kini A-D, Firestein S. 2000. The molecular receptive range of an odorant receptor. *Nature Neuroscience*

Araneda R-C, Peterlin Z, Zhang X, Chesler A, Firestein S. 2004. A pharmacological profile of the aldehyde receptor repertoire in rat olfactory epithelium. *Journal of Physiology* 555: 743-56

Arn HaTA. 1998. Flavornet: "a database of aroma compounds based on odor potency in natural products.". http://www.flavornet.org

Arohnsohn E. 1886. Experimentelle Untersuchungen zur Physiologie des Geruchs. *Arch. Anat. Physiol. physiol. Abt.*: 320-57

Aungst B. 2003. Predicting poor permeability and formulation approaches to overcome it. *Journal of Pharmacy and Pharmacology* 55

Aungst J, Heyward P, Shipley MT. 2003a. Evidence for dendritic localization of Ih in granule cells of mouse main olfactory bulb. *Society for Neuroscience Abstract Viewer and Itinerary Planner* 2003

Aungst JL, Heyward PM, Puche AC, Karnup SV, Hayar A, et al. 2003b. Centre-surround inhibition among olfactory bulb glomeruli. *Nature (London)* 426: 623-9

Axel R. 1995. The molecular logic of smell. *Scientific American*

Backman EL. 1917a. Note sure la puissance des odeurs et leur solubilite dans l'eau et dan l'huile. *Jour. Physiol. Pathol. Gen.*: 1-4

Backman EL. 1917b. Olfactology of the Methyl-benzene Series. *Proc. Akad. Wetensch* 19: 943-56

Backman EL. 1917c. Ueber di Versataubungselektrictat. *Arch. ges. Physiol.* 168: 351-71

Bartoshuk LM. 1975. Taste Mixtures Is Mixture Suppression Related To Compression. *Physiology and Behavior*

Baylis GC, Driver J. 2001. Shape-coding in IT cells generalizes over contrast and mirror reversal, but not figure-ground reversal. *Nature (London)* 4: 937-42

Beck J, Rosenfeld A, Ivry R. 1989. Line Segregation. *Spatial Vision* 4: 75-101

Bell GA, Laing DG, Panhuber H. 1987. Odor Mixture Suppression Evidence For A Peripheral Mechanism In Human And Rat. *Brain Research*

Berglund B, Berglund U, Engen T, Lindvall T. 1971. The Effect Of Adaptation On Odor Detection. *Perception and Psychophysics*

Berglund B, Berglund U, Thomas L. 1976. Psychological processing of odor mixtures. *Psychological Review* 83: 432-41

Berglund B, Olsson M-J. 1993a. Odor-intensity interaction in binary and ternary mixtures. *Perception and Psychophysics*

Berglund B, Olsson M-J. 1993b. Odor-intensity interaction in binary mixtures. *Journal of Experimental Psychology Human Perception and Performance*

Best AR, Wilson DA. 2004. Coordinate synaptic mechanisms contributing to olfactory cortical adaptation. *Journal of Neuroscience* 24: 652-60

Biederman I. 1987. Recognition-by-components: a theory of human image understanding. *Psychological Review* 94: 115-47

Buck L, Axel R. 1991. A Novel Multigene Family May Encode Odorant Receptors a Molecular Basis for Odor Recognition. *Cell*

Cain W-S, Polak E-H. 1992. Olfactory adaptation as an aspect of odor similarity. *Chemical Senses*

Cain W-S, Schmidt R. 2002. Sensory detection of glutaraldehyde in drinking water-emergence of sensitivity and specific anosmia. *Chemical Senses*

Cain WS. 1971. Physicochemical Characteristics and Supraliminal Odor Intensity Reply to Mitchell. *Perception and Psychophysics* 9: 478-9

Cain WS. 1974. Contribution of the Trigeminal Nerve to Perceived Odor Magnitude. *Annals of the New York Academy of Sciences* 237: 28-34

Cain WS, Drexler M. 1974. Odors: Evaluation, Utilization, and Control. *Ann N Y Acad Sci* 237: 427-39

Cain WS, Engen T. 1969. Olfactory Adaptation and the Scaling of Odor Intensity. In *Pfaffman, Carl (Ed.). Olfaction and Taste. Symposium. Xiii+648p. Illus. Rockefeller University Press: New York, N.Y., U.S.A*, pp. 128-41

Cheesman GH, Mayne S. 1953. The influence of adaptation on absolute threshold measurements for olfactory stimuli. *The Quarterly Journal of Experimental Psychology* 5: 22-30

Chess A, Simon I, Cedar H, Axel R. 1994. Allelic inactivation regulates olfactory receptor gene expression. *Cell*

Colbert HA, Bergmann CI. 1995. Odorant-Specific Adaptation Pathways Generate Olfactory Plasticity in C. elegans. *Neuron* 14: 803-12

Cometto-Muniz J-E, Cain W-S, Abraham M-H. 2005. Odor detection of single chemicals and binary mixtures. *Behavioral Brain Research*

Cometto-Muniz J-E, Cain W-S, Abraham M-H, Gola J-M-R. 1999. Chemosensory detectability of 1-butanol and 2-heptanone singly and in binary mixtures. *Physiology and Behavior*

Dalton P. 2000. Psychophysical and Behavioral Characteristics of Olfactory Adaptation. *Chemical Senses* 25: 487-92

Dalton P, Wysocki CJ. 1996. The nature and duration of adaptation following long-term odor exposure. *Perception and Psychophysics* 58: 781-92

Derby C-D, Hutson M, Livermore B-A, Lynn W-H. 1996. Generalization among related complex odorant mixtures and their components: Analysis of olfactory perception in the spiny lobster. *Physiology and Behavior*

Derby CD, Gleeson RA, Ache BW. 1984. Peripheral And Central Events Contribute To Mixture Suppression In The Olfactory Pathway. *Society for Neuroscience Abstracts*

Duchamp-Viret P, Chaput M-A, Duchamp A. 1999. Odor response properties of rat olfactory receptor neurons. *Science (Washington D C)*

Edelman GM. 2004. *Wider than the Sky*. New Haven: Yale University Press

Elsberg CA, Levy I, Brewer ED. 1935. The sense of smell VI. The trigeminal effects of odorous substances. *Bull. Neurol. Inst. N.Y.* 4: 270-85

Engen T. 1962. *The psychophysical similarity of the odors of aliphatic alcohols*, University of Stockholm

Engen T. 1964a. *Direct scaling of odor intensity.*, University of Stockholm

Engen T. 1964b. Psychophysical scaling of odor intensity and quality. *Ann N Y Acad Sci* 116: 504-16

Firestein S. 2001. How the olfactory system makes sense of scents. *Nature (London)*

Firestein S, Shepherd GM. 1990. The Role of Cyclic Amp as a Second Messenger in Vertebrate Olfactory Transduction. *Chemical Senses* 15

Firestein S, Shepherd GM, Werblin FS. 1990. Time Course of the Membrane Current Underlying Sensory Transduction in Salamander Olfactory Receptor Neurons. *Journal of Physiology (Cambridge)* 430: 135-58

Friedrich R-W, Korsching SI. 1997. Combinatorial and chemotopic odorant coding in the zebrafish olfactory bulb visualized by optical imaging.

Neuron 18: 737-52

Fuss SH, Korsching SI. 2001. Odorant feature detection: activity mapping of structure response relationships in the zebrafish olfactory bulb. *Journal of Neuroscience* 21: 8396-407

Gescheider Ga. 1997. *Psychophysics: The Fundamentals*. Mahwah: Lawrence Erlbaum Associates. 435 pp.

Goldstein BE. 2001. *Sensation and Perception*. Belmont, CA: Wadsworth Publishing Company. 704 pp.

Gottfried J-A, Winston J-S, Dolan R-J. 2006. Dissociable codes of odor quality and odorant structure in human piriform cortex. *Neuron*

Goyert H-F, Frank M-E, Gent J-F, Hettinger T-P. 2007. Characteristic component odors emerge from mixtures after selective adaptation. *Brain Research Bulletin*

Harper R, Bate Smith EC, Laud DG. 1967. *Odour Description and Odour Classification: A multidisciplinary examination*. New York: American Elsevier Publishing Company Inc.

Hellman, A., Chess, A. 2002. Olfactory Axons: A remarkable Convergence. *Current Biology.* 24. 849-851.

Hermanides J. 1909. *Over de constanten der in der olfaktomertrie gebruikelijke negen standaardgeuren*. University of Utrecht

Heymann HH. 1994. A comparison of descriptive analysis of vanilla by two independently trained panels. *Journal of Sensory Studies* 9: 21-32

Huang YT, Lawless HT. 1998. Sensitivity of the ABX Discrimination Test. *Journal of Sensory Studies* 13: 229-39

Hubel D, H., Wiesel T, N. 1959. Receptive fields of single neurons in the cats striate cortex. *The Journal of Physiology* 148: 574-91

Hubel D, H., Wiesel T, N. 1962. Receptive fields, binocular interaction and functional architecture in the cats visual cortex. *The Journal of Physiology* 147: 226-38

Jinks A, Laing DG. 1999. Temporal processing reveals a mechanism for limiting the capacity of humans to analyze odor mixtures. *Cognitive Brain Research* 8: 311-25

Jones FN, Woskow MH. 1964. On the intensity of odor mixtures [man]. *Ann N Y Acad Sci* 116: 484-94

Julesz B. 1981. Textons, the elements of texture perception, and their interactions. *Nature (London)* 290: 91-7

Julesz B. 1984. A brief outline of the texton theory of human vision. *Trends in Neuroscience* 7: 41-5

Kadohisa M, Wilson D-A. 2006. Olfactory cortical adaptation facilitates detection of odors against background. *Journal of Neurophysiology (Bethesda)*

Kajiya K, Inaki K, Tanaka M, Haga T, Kataoka H, Touhara K. 2001. Molecular bases of odor discrimination: Reconstitution of olfactory receptors that recognize overlapping sets of odorants. *Journal of Neuroscience*

Kandel ER, Schwartz JH, Jessell TM. 2000. *Principles of Neural Science*. New York: McGraw-Hill

Kay L-M, Crk T, Thorngate J. 2005. A redefinition of odor mixture quality. *Behavioral Neuroscience*

Kay L-M, Lowry C-A, Jacobs H-A. 2003. Receptor contributions to configural and elemental odor mixture perception. *Behavioral Neuroscience*

Keller A, Zhuang H, Chi Q, Vosshall LB, Matsunami H. 2007. Genetic Variation in a human odorant receptor alters odour perception. *Nature*

(London) 449: 468-72

Kittel KM, Barnard J, Kurtz AJ, Acree TE. 2008. Free-Choice Profiling of OR-I7 Agonists and Homolgues Using GC-O. *Chemsensory Perception*: 1-15

Koster EP. 1965. Olfactory sensitivity and the menstrual cycle. *Int Rhin.* 1: 57

Koster EP. 1968. Olfactory sensitivity and ovulatory cycle duration. *Olfactologia* 1: 43

Koster EP. 1971. *Adaptation and Cross-adaptation in olfaction: an experimental study with olfactory stimuli at low levels of intensity*. Doctoral Dissertation thesis. University of Utrecht

Kunnupas T. 1957. Experiments on figural dominance. *Journal of Experimental Psychology* 53: 31-9

Kurahashi T, Shibuya T. 1990. Calcium Dependent Adaptive Properties in the Solitary Olfactory Receptor Cell of the Newt. *Brain Research* 515: 261-8

Laing DG. 1986. Identification of Single Dissimilar Odors Is Achieved by Humans with a Single Sniff. *Physiology and Behavior* 37: 163-70

Laing DG, Eddy A, Francis GW, Stephens L. 1994. Evidence for the temporal processing of odor mixtures in humans. *Brain Research* 651: 317-28

Laing DG, Francis GW. 1989. The Capacity Of Humans To Identify Odors In Mixtures. *Physiology and Behavior*

Laing DG, Panhuber H, Willcox ME, Pittman EA. 1984. Quality And Intensity Of Binary Odor Mixtures. *Physiology and Behavior*

Laing DG, Wilcox ME. 1983. Perception Of Components In Binary Odor Mixtures. *Chemical Senses*

Laing DG, Willcox ME. 1987. An Investigation of the Mechanisms of Odor Suppression Using Physical and Dichronic Mixtures. *Behavioral Brain Research* 26: 79-88

Lancet D, Sadovsky E, Seidemann E. 1993. Probability model for molecular recognition in biological receptor repertoires: significance to the olfactory system. *Proc. Natl. Acad. Sci.* 90: 3715-9

Laska M, Hudson R. 1993. Discriminating parts from the whole: Determinants of odor mixture perception in squirrel monkeys, Saimiri sciureus. *Journal of Comparative Physiology A Sensory Neural and Behavioral Physiology*

Laska M, Teubner P. 1999. Olfactory Discrimination Ability for Homologous Series of Aliphatic Alcohols and Aldehydes. *Chemical Senses* 24: 263-70

Lawless HT. 1987. An Olfactory Analogy To Release From Mixture Suppression In Taste. *Bulletin of the Psychonomic Society*

Lawless HT. 1989. Exploration of fragrance categories and ambiguous odors using multidimensional scaling and cluster analysis. *Chemical Senses* 14: 349-60

Lawless HT, Heymann HH. 1998. *Sensory Evaluation of Food*. New York: Springer

Lawless HT, Sheng N, Knoops SSCP. 1995. Multidimensional scaling of sorting data applied to cheese perceptions. *Food Quality and Preference* 6: 91-8

Lawless HT, Stevens DA. 1983. Cross Adaptation Of Sucrose And Intensive Sweeteners. *Chemical Senses*

Leffingwell JC, Leffingwell D. 1991. GRAS Flavor Chemicals-- Detection Thresholds. *Perfumer & Flavorist* 16: 1-19

Levasseur G, Persuy M-A, Grebert D, Remy J-J, Salesse R, Pajot-Augy E. 2003. Ligand-specific dose-response of heterologously expressed olfactory receptors. *European Journal of Biochemistry*

Linster C, Cleland T-A. 2004. Configurational and elemental odor mixture perception can arise from local inhibition. *Journal of Computational Neuroscience*

Linster C, Smith BH. 1999. Generalization Between Binary Odor Mixtures and Their Components in the Rat. *Physiology and Behavior* 66: 701-7

Livermore A, Laing D-G. 1996. Influence of training and experience on the perception of multicomponent odor mixtures. *Journal of Experimental Psychology Human Perception and Performance*

Livermore A, Laing D-G. 1998. The influence of odor type on the discrimination and identification of odorants in multicomponent odor mixtures. *Physiology and Behavior*

Macmillan NA, Creelman D. 2005. *Detection Theory: A User's Guide*. Mahwah, NJ: Lawrence Erlbaum Associates

Macmillan NA, Kaplan J, Creelman C. 1977. The psychophysics of categorical perception. *Psychological Review* 94: 452-71

Malnic B, Hirono J, Sato T, Buck L-B. 1999. Combinatorial receptor codes for odors. *Cell*

Man, O., Gilad, Y., Lancet, D. Prediction of the odorant binding site of the olfactory receptor proteins in human-mouse comparison. *Protein Science.* 13: 240-254.

Mandairon N, Stack C, Linster C. 2006. Olfactory enrichment improves the recognition of individual components in mixtures. *Physiology and Behavior*

Marin AB, Barnard J, Darlington RB, Acree TE. 1991. Sensory thresholds: estimation from dose-response curves. *Journal of Sensory Studies* 6:

205-25

Matarazzo M, Giardina MG, Guardasole V, Davalli AM, Horton ES, et al. 2002. Islet transplantation under the kidney capsule corrects the defects in glycogen metabolism in both liver and muscle of streptozocin-diabetic rats. *Cell Transplantation* 11: 103-12

Menashe I, Man O, Lancet D, Gilad Y. 2003. Different noses for different people. *Nature Genetics*

Mombaerts P. 1999. Seven-transmembrane proteins as odorant and chemosensory receptors. *Science (Washington D C)*

Mombaerts P. 2004. Genes and ligands for odorant, vomeronasal and taste receptors. *Nature Reviews Neuroscience*

Mombaerts P, Wang F, Dulac C, Chao S-K, Nemes A, et al. 1996. Visualizing an olfactory sensory map. *Cell*

Mori K, Nagao H, Yoshihara Y. 1999. The olfactory bulb: Coding and processing of odor molecule information. *Science (Washington D C)*

Morrison EE, Costanzo RM. 1990. Morphology of the Human Olfactory Epithelium. *Journal of Comparative Neurology*

Mozell MM. 1970. Evidence for a Chromatographic Model of Olfaction. *Journal of General Physiology*

Nagata Y, Takeuchi N. 1990. Determination of odor threshold value by triangle odor bag method. *Bulletin of Japan Environmental Sanitation Center* 17: 77-89

Nagel W. 1904. Der Geruchassinn. *Handb. Physiol. Menschen* 3: 589-620

Oyama T. 1960. Figure-ground dominance as a function of sector angle, brightness, hue, and orientation. *Journal of Experimental Psychology* 60: 299-305

Parker GH. 1922. *Smell, Taste, and Allied Senses in the Vertebrates*. Philadelphiaa: J.B. Lippincott Company. 198 pp.

Paysan J, Breer H. 2001. Molecular physiology of odor detection: Current views. *Pfluegers Archiv European Journal of Physiology*

Peterson MA. 1994. Object recognition processes can and do operate before figure-ground organization *Current Directions in Psychological Science* 3: 105-11

Pevsner J, Hwang PM, Sklar PB, Venable JC, Snyder SH. 1988a. Odorant-Binding Protein and Its Messenger Rna Are Localized to Lateral Nasal Gland Implying a Carrier Function. *Proceedings of the National Academy of Sciences of the United States of America* 85: 2383-7

Pevsner J, Reed RR, Feinstein PG, Snyder SH. 1988b. Molecular Cloning of Odorant-Binding Protein Member of a Ligand Carrier Family. *Science (Washington D C)* 241: 336-9

Pierce JD, Wysocki CJ, Aronov EV, Webb JB, Boden RM. 1996. The Role of Perceptual and Structural Similarity in Cross-adaptation. *Chemical Senses* 21: 223-37

Pierce JR, Gilbert EN. 1958. On AX and ABX Limens. *The Journal of the Acoustical Society of America* 30: 593-5

Pilpel Y, Lancet D. 1999. The variable and conserved interfaces of modeled olfactory receptor proteins. *Protein Science* 8: 969-77

Rabin M-D, Cain W-S. 1984. Odor recognition: familiarity, identifiablility, and encoding consistency. *Journal of Experimental Psychology: Learning, Memory, and Cognition*

Rawson N-E, Eberwine J, Dotson R, Jackson J, Ulrich P, Restrepo D. 2000. Expression of mRNAs encoding for two different olfactory receptors in a

subset of olfactory receptor neurons. *Journal of Neurochemistry*
Ressler KJ, Sullivan SL, Buck L. 1994. Information coding in the olfactory system: Evidence for a stereotyped and highly organized eptiope map in the olfactory bulb. *Cell* 79: 1245-55
Rubin N, Katz LC. 1999. Optical imaging of odorant representations in the mammalian olfactory bulb. *Neuron* 23: 499-511
Serizawa S, Miyamichi K, Nakatani H, Suzuki M, Saito M, et al. 2003. Negative feedback regulation ensures the one receptor-one olfactory neuron rule in mouse. *Science (Washington D C)*
Shepherd GM. 2006. Smell images and the flavour system in the human brain. *Nature (London)* 444: 316-21
Singer MS. 2000. Analysis of the Molecular Basis of Octanal Interactions in the Expressed Rat I7 Olfactory Receptor. *Chemical Senses* 25: 155-65
Small D. 2004. Crossmodal integration- insights from the chemical senses. *Trends in Neuroscience* 27: 120-4
Small D, Voss J, Mak Y, Simmons K, Parrish T, Gitelman D. 2004. Experience-dependent neural integration of taste and smell in the brain. *Journal of Neurophysiology (Bethesda)* 92: 1892-903
Smith BH. 1996. The role of attention in learning about odorants. *Biol. Bull* 191: 76-83
Spehr M, Gisselmann G, Poplawski A, Riffell JA, Wetzel CH, et al. 2003. Identification of a testicular odorant receptor mediating human sperm chemotaxis. *Science (Washington D C)* 299: 2054-8
Staubli U, Schottler F, Nejat-Bina D. 1987. Role of dorsomedial thalamic nucleus and piriform cortex in processing olfactory information. *Behavioral Brain Research* 25: 117-29

Stevenson R-J. 2001. Associative learning and odor quality perception: How sniffing an odor mixture can alter the smell of its parts. *Learning and Motivation*

Stevenson R-J, Case TI, Tomiczek C. 2007. Resistance to interference of olfactory perceptual learning. *The Psychological Review* 57: 103-16

Stewart WB, Kauer JS, Shepherd GM. 1979. Functional organization of rat olfactory bulb analyzed by the 2-deoxyglucose method. *Journal of Comparative Neurology* 185: 715-34

Stone H, Pryor GT, Steinmetz G. 1972. A Comparison of Olfactory Adaptation among 7 Odorants and Their Relationship with Several Physicochemical Properties. *Perception and Psychophysics* 12: 501-4

Stuiver M. 1958. *Biophysics of the Sense of Smell*. Rijks University, Groningen, The Netherlands

Tabor R, Yaksi E, Weislogel J-M, Friedrich R-W. 2004. Processing of odor mixtures in the zebrafish olfactory bulb. *Journal of Neuroscience*

Tinoco I, Sauer, K., and Wang, J. 1995. *Physical chemistry: principles and applications in biological sciences.* Englewood Cliffs.: Prentice-Hall Inc.

Todrank J, Wysock CJ, Beauchamp GK. 1991. The Effects Of Adaptation On The Perception Of Similar And Dissimilar Odors. *Chemical Senses*

Torre V, Ashmroe JF, Lamb TD, Menini A. 1995. Transduction and adaptation in sensory receptor cells. *Journal of Neuroscience* 15: 7757-68

Touhara K. 2008. Structure, expression, and function of olfactory receptors. In *The Senses; a comprehensive reference*, ed. S Firestein, GK Beauchamp, pp. 527-44. Amsterdam: Elsevier

Treisman A, Gormican S. 1988. Feature analysis in early vision: evidence from search asymmetries. *Psychological Review* 95: 15-48

Vaschide N. 1901. L'experience de Wever et l'olfaction en milieu liquide. *Compt. rend. Soc. Biol.* 53

Vassar R, Chao S-K, Sitcheran R, Nunez JM, Vosshall LB, Axel R. 1994. Topographic organization of sensory projections to the olfactory bulb. *Cell* 79: 981-91

Vassar R, Ngai J, Axel R. 1993. Spatial segregation of odorant receptor expression in the mammalian olfactory epithelium. *Cell* 74: 309-18

Vecera SP, O'Reilly RC. 2000. Graded effects in hierarchical figure-ground organization: reply to Peterson (1999). *Journal of Experimental Psychology: Human Perception and Performance* 26: 1221-31

Wachowiak M, Cohen L. 2001. Representation of odorants by receptor neuron input to the mouse olfactory bulb. *Neuron* 32: 723-35

Wiesel T, N., Hubel D, H. 1963. Single-cell responses in striate cortex of kittens deprived of vision in one eye. *Journal of Neurophysiology (Bethesda)* 26: 1003-17

Wilson DA. 1998. Synaptic correlates of odor habituation in the rata anterior piriform cortex. *Journal of Neurophysiology (Bethesda)* 80: 998-1001

Wiltrout C, Dogra S, Linster C. 2003. Configurational and nonconfigurational interactions between odorants in binary mixtures. *Behavioral Neuroscience*

Wuttke MS, Tompkins L. 2000. Olfactory Adaptation in Drosphilia Larvae. *Journal of Neurogenetics* 14: 43-62

Yadon CA, Wilson DA. 2005. The role of metabotropic glutamate receptors and cortical adaptation in habituation of odor-guided behavior. *Learning & Memory (Cold Spring Harbor)* 12: 601-5

Yantis S, ed. 2001. *Visual Perception: Essential Readings*. Philadelphia, PA:

Psychology Press. 431 pp.

Zhao H, Ivic L, Otaki J-M, Hashimoto M, Mikoshiba K, Firestein S. 1998. Functional expression of a mammalian odorant receptor. *Science (Washington D C)*

Zou Z, Buck L-B. 2006. Combinatorial effects of odorant mixes in olfactory cortex. *Science (Washington D C)*

Zwaardemaker H. 1895. Die Physiologie des Geruchs. *Leipzig*: 324

Zwaardemaker H. 1920. On Physiological Radio-activity. *Jour. Physiol.* 53: 273-89

CHAPTER 2

OBJECTIVES AND HYPOTHESES

2.1 OBJECTIVES

This research has the following objectives (Figure 2.1)

1. Examine the perceptual properties of straight chain aldehyde homologues C_6, C_8, C_{10}, C_{11} based on evidence provided by the physiological readings of rat olfactory receptor I7 (Zhao et al 1998).
2. Determine range of detection of odorants C_6, C_8, C_{10}, and C_{11} to create dose-response curves using a 3-alternative, forced-choice (3-AFC) method used in Cometto-Muniz et. al. (2005) (Cometto-Muniz et al 2005).
3. Create a method to test panelist's ability to discriminate between low, medium, and high intensity within an odorant set using an odor-reference matching tasks.
4. Devise a method to measure perceptual changes in intensity resulting from adaptation and cross adaptation.
5. Use the resulting panel to examine psychophysically, the cross-adaptation of odorants C_6, C_8, C_{10}, and C_{11}, to study the influence of chemical functionality and odorant similarity on cross-adaptation.
6. Develop a method to measure perceptual changes of odor components within binary mixtures, by creating figure-ground matching tasks through the use of figure-references.
7. Psychophysically evaluate mixture perception using a panel.
8. Gain further insight into the influence of OR-ligand interaction on perceptions.

A flow chart outlining the experimental process is presented in Figure 2.1.

2.2 HYPOTHESES

This research has the following hypotheses:

1. Based on the physiological recordings of OR-I7 and homologues C_6-C_{11} as well as FCP, C_6 will not cross-adapt with C_8, C_{10}, or C_{11}. However, cross-adaptation will be greatest for odorants C_8 and C_{10} due to their odor quality similarity as well as their high activation of OR-I7. Cross-adaptation will also occur between C_{11} and C_8 and C_{10} due to odorant quality similarity.
2. In binary solutions of dissimilar odor quality and dissimilar OR activation, panelists will be able to distinguish the figure odorant from the ground depending on the ratios of the figure-ground intensities within the mixture. Figure suppression will occur as the ground odorant becomes of equal or greater intensity than the figure odorant.

EXPERIMENT 1

The purpose of the first experiment was to examine the relationship between ligand-OR binding and the relationship between odor quality and cross-adaptation. Odorants C_6, C_8, C_{10}, and C_{11} were chosen as a homologues series known for their ability and inability to excite OR-I7 in the rat mammalian model. Experiments were designed to examine the relationship between cross-adaptation and the published ligand-OR relationships of OR-I7 as well as the relationship between odor quality similarity and cross-adaptability (Cain & Polak 1992, Todrank et al 1991). Low, medium, and high intensities for all four odorants was determined through a 3-alternative, forced-choice (3-AFC) tests where subjects selected the bottle containing an odorant, and evaluated their confidence on a 5-point scale and it's intensity on a 9-point scale (Cometto-Muniz et al 2005). This method allowed for the determination of odorant detection intensities for all odorants as well as subjects to effectively rate odorants for confidence of detection as well as intensity level with multiple replicates for each intensity level evaluated.

The method used for evaluating intensity changes due to adaptation was based upon the work of Todrank et. al.(1991) and Lawless (1987) (Lawless 1987, Todrank et al 1991). Subjects used references to evaluate changes in intensity during a prior training session. Four different conditions were used to test for adaptation: control, scaling, self-adaptation, and cross-adaptation. Three of the conditions allowed the researcher to check the effectiveness of scale usage as well the ability of the panelist to adapt. Panelists evaluated the cross-adaptability of the three citrus odorants and one green odorant.

EXPERIMENT 2

The purpose of Experiment 2 was to determine whether an individual is capable of detecting elemental components of a binary mixture within a binary mixture using dissimilar odorants while varying the ratios of the intensities of the two odorants in the mixture. Several studies have determined dissimilar odorants are more easily perceived within a mixture (Goyert et al 2007, Laing 1986, Livermore & Laing 1998) this experiment further examined the ability of an individual to perceive intensities of odorants within a mixture using a odor reference matching task similar to a AB-X test. However, three references were used instead of two, thus A, B, and C. Additionally, instead of A, B, and

C being different stimuli A, B, and C were varying intensities of the same odorant. A: PEG (solvent), B: Medium intensity of the odorant C: High intensity of the odorant. Subjects were trained to properly match individual odorants to their appropriate intensity reference. Using a reference-matching task eliminates the need for subjects to quantitatively rate odorants, a task requiring subjects to remember odor intensity, an extremely difficult and unreliable task. Odorants C_6 and C_8 were chosen due to their dissimilar odorant quality as determined by Kittel et. al. (2008) as well as their inability to cross-adapt as shown in experiment 1.

There were two different types of ABC-X matching tasks: a C_6 figure matching task, and a C_8 figure-matching task. The figure in the binary mixture is defined by the odorant chosen as the reference. If the reference is C_6 subjects are asked to match the intensity of the mixture based on the perceived intensity of C_6 within the mixture. Panelists were presented with mixtures of C_6 and C_8 of varying intensities. Panelists were asked to differentiate the odorants within the mixture and determine the intensity of the figure odorant within the mixture to the reference of similar intensity. This method allows for the investigation of the influence of the ratio of the intensities within the mixture to influence the ability to perceive the figure odorant within the mixture.

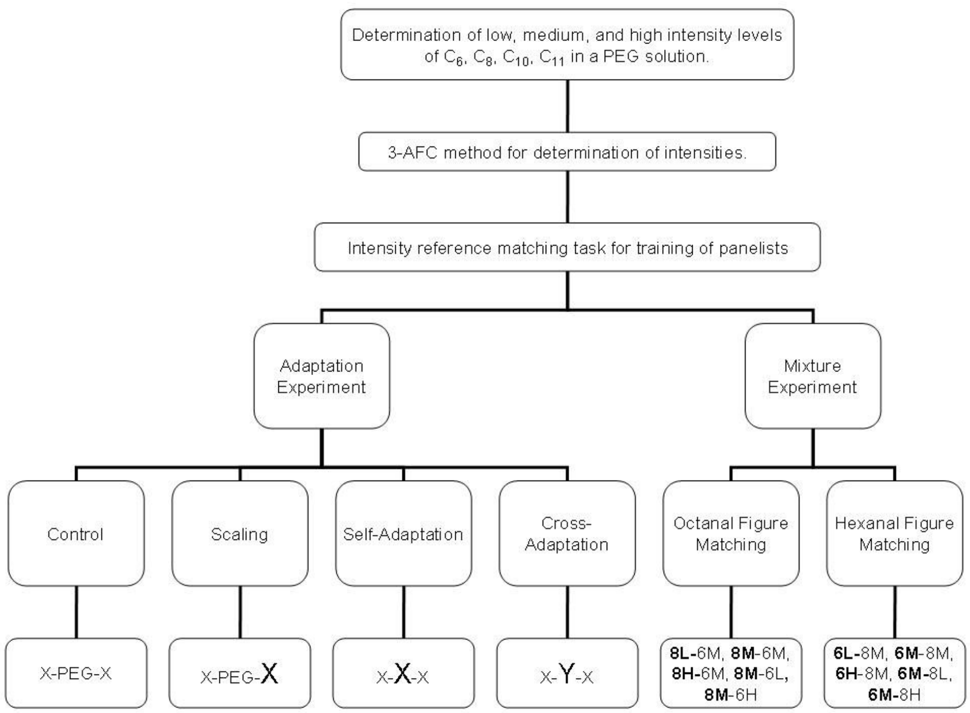


Figure 2.1: Flow chart for experimental design. X represents a medium intensity of an odorant. PEG is Poly(ethylene glycol) the solvent. X high intensity of an odorant. Y is a high intensity of a different odorant. Stimuli labeled in bold are the odorants defined as figure in the matching task.

REFERENCES

Cain W-S, Polak E-H. 1992. Olfactory adaptation as an aspect of odor similarity. *Chemical Senses*

Cometto-Muniz J-E, Cain W-S, Abraham M-H. 2005. Odor detection of single chemicals and binary mixtures. *Behavioral Brain Research*

Goyert H-F, Frank M-E, Gent J-F, Hettinger T-P. 2007. Characteristic component odors emerge from mixtures after selective adaptation. *Brain Research Bulletin*

Laing DG. 1986. Identification of Single Dissimilar Odors Is Achieved by Humans with a Single Sniff. *Physiology and Behavior* 37: 163-70

Lawless HT. 1987. An Olfactory Analogy To Release From Mixture Suppression In Taste. *Bulletin of the Psychonomic Society*

Livermore A, Laing D-G. 1998. The influence of odor type on the discrimination and identification of odorants in multicomponent odor mixtures. *Physiology and Behavior*

Todrank J, Wysock CJ, Beauchamp GK. 1991. The Effects Of Adaptation On The Perception Of Similar And Dissimilar Odors. *Chemical Senses*

Zhao H, Ivic L, Otaki J-M, Hashimoto M, Mikoshiba K, Firestein S. 1998. Functional expression of a mammalian odorant receptor. *Science (Washington D C)*

CHAPTER 3

EXPERIMENT 1: CROSS-ADPATATION BETWEEN OR-I7 AGONISTS AND HOMOLOGUES

3.1 MATERIALS & METHODS

3.1.1 *PANELISTS*

Six panelists, five women and one man, non-smokers with normal olfactory function and mean age of 25.8 (S.D. 4.2) years, volunteered to participate. All panelists were non-smokers. The protocol was reviewed and approved by the Institutional Review Board of Cornell University. All subjects were paid for their participation.

3.1.2. *STIMUL*I

Straight chain aliphatic aldehydes C_6, C_8, C_{10}, and C_{11} were obtained from Sigma-Aldrich (ST. Louis, MO) and were reagent grade: Hexanal (98%), octanal (99%), decanal (99%), undecanal (97%), poly(ethylene glycol)

Solvent: Poly(ethylene glycol) (PEG) Medium and high intensity dilutions were made of all four stimuli (Table 3.1). Medium intensity component stimuli were: 7.5 mM $C_{6,}$ 3 mM C_8, 5 mM $_{C10}$, 2 mM C_{11}. High intensity component stimuli were: 120 mM C_6, 24 mM C_8, 20 mM C_{10}, and 18 mM $C_{11.}$ All odorants were presented in 250 mL polyethylene squeeze bottles similar to those used in by Goyert (2007). Bottles were modified with 1.5 cm Teflon balls fitted around the neck of the bottle for nasal comfort (Fig. 3.1). A hole was drilled through the center of a teflon ball using a size G drill bit (0.2610 diameter inches), in order to properly fit on the neck of the bottle. Stimuli were presented on perfumer's blotters (Frank Orlandi, 'Red Line') dipped in PEG based stimuli to 1 cm then placed on the plastic squeeze bottles and left to

Table 3.1: Stimuli concentrations in mM of low and medium intensity odorants.

Odorant	C_6	C_8	C_{10}	C_{11}
Middle Intensity	7.5	3	5	2
High Intensity	120	24	20	18

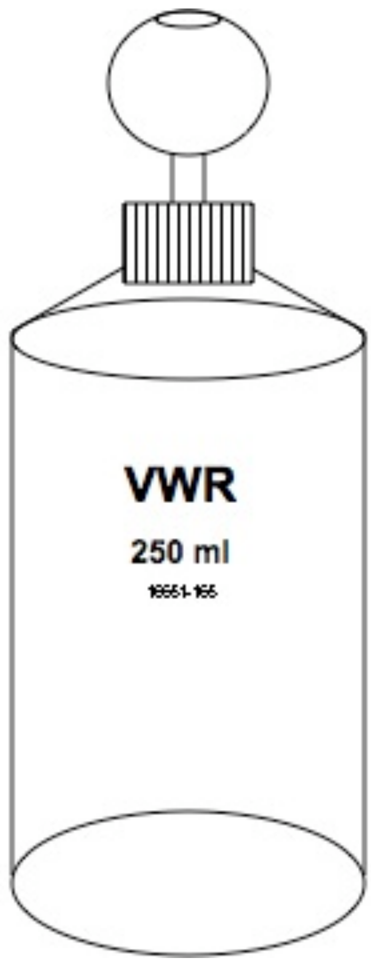


Figure 3.1: Modified plastic bottle for odorant delivery.

equilibrate for one hour prior to testing. All bottles were labeled with random three-digit codes. Prior to testing, panelists were instructed on where to hold the bottles in front of their face, and how to breathe when squeezing the bottles, to ensure proper stimulus delivery.

3.1.3. *ODOR INTENSITY DETERMINATION*

Odorant intensity determination followed a 3-alternative forced choice (AFC) method outlined by Cometto-Muniz et. al. (2005) (Cometto-Muniz et al 2005). Panelists were presented with three bottles in random order and asked to indicate which bottle contained the odorant. In all conditions, panelists received two bottles containing PEG, and one bottle containing odorant. Order of presentation was randomized throughout the presentation process. Panelists were presented with six different concentrations of each of the four odorants. Middle and high intensities of each odorant were determined based on the dose-response curves produced from the results. Once the panelist indicated which bottle contained the odorant, panelists made an intensity judgment based on a 9 point scale, where 1= no odor and 9 = very strong. Additionally panelists rated their confidence for each judgment of the presence of the odorant by using a 5 point judgment scale where 1= not confident and 5= very confident. All odorants were chosen to be within the detectible range.

3.1.4 *TRAINING*

Nine panelists initially participated in the training session. Six panelists were chosen to be in the study and trained to distinguish between the solvent (PEG), middle intensity, and high intensity of each odorant (the only concentrations to be tested). Panelists were trained in individual sessions for each of the four odorants, C_6, C_8, C_{10}, and C_{11}. Each training session lasted approximately 10 minutes. In each session panelists were asked to evaluate the intensities of seven test bottles, with a 30 second break between each bottle. Panelists used a modified ABC-X task described below to evaluate the intensities of the seven bottles. Upon completion of evaluating the seven bottles, panelists were informed of their performance. After a two-minute break, panelists were asked to evaluate a new set of seven test bottles through the same process. A modified AB-X sorting method was used for training called the ABC-X reference-matching task. An AB-X sensory test allows panelists to match stimuli to two different references A and B (Cometto-Muniz et al 2005, Huang & Lawless 1998, Lawless & Heymann 1998, Macmillan & Creelman 2005). Figure 3.3 illustrates the reference-matching task used for training.

The references in the ABC-X task were PEG (A), medium intensity (B), and high intensity (C). Panelists were instructed to smell references A, B, C and familiarize themselves with these three intensities. Once panelists were familiar with the three reference intensities, panelists were asked to sort seven randomly placed test bottles into the appropriate intensity groups. Six bottles contained two bottles of PEG, two bottles of medium intensity, and two bottles of high-intensity. One extra bottle either of PEG, medium intensity, or high intensity was added to the six bottles for a total of seven bottles. The extra bottle was used to ensure the panelist did not use a process-of-elimination to sort the bottles. An example of a C_6 reference-matching task is outlined in figure 3.2. Reference A was PEG, reference B (medium intensity) was 7.5 mM, and C (high intensity) was 120 mM. In this example there are seven test-bottles, where two bottles are marked PEG, three test bottles are marked M, and two test bottles are marked H. The panelist's task is to place each bottle of unmarked test-bottles in front of the

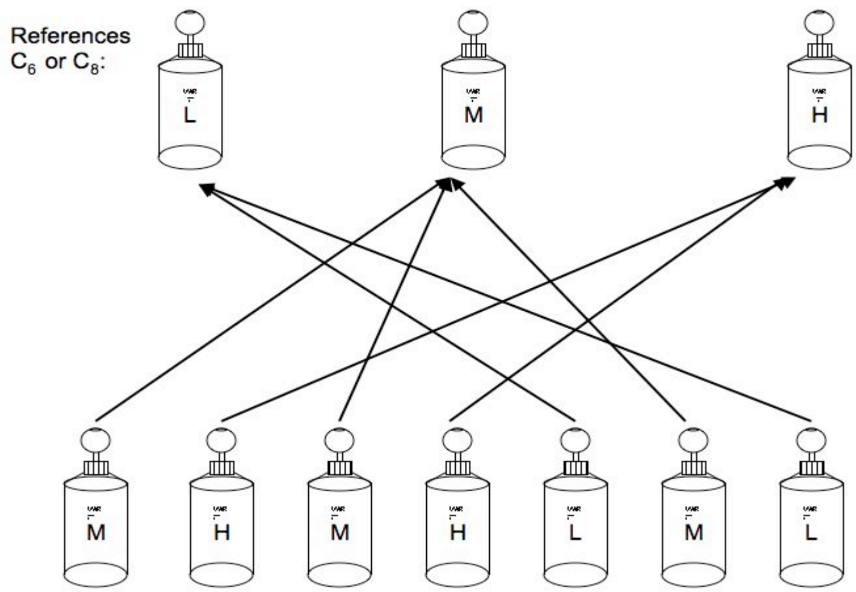


Figure 3.2: Odor reference matching task used for training. The three bottles at the top are the references: low intensity (L) medium intensity (M), and high intensity (H). All reference bottles contain the same odorant. Either C_6 or C_8. If the reference bottles contain C_6 the test-bottles would contain C_6. The seven bottles at the bottom of the figure are test-bottles containing L, M, or H intensity of the same odorant as the reference. The panelist must place the test-odor bottle in front of the reference bottle of the same intensity. The arrows indicate the proper placement of the test-bottle to the reference of the same intensity.

matching reference bottle. Once complete, the panelist alerted the researcher. The researcher checked the placement of the bottles and alerted the panelist of any errors. If there were errors, the panelist was asked to re-evaluate the bottle. In order for a panelist to receive a score of 100%, the panelist was required to properly match all test-bottles to the corresponding reference bottle. The panelist had to complete this task correctly on two consecutive sessions in order to continue onto to the adaptation test. Obtaining a score of 100% in training indicated the panelist's ability to detect the presence of the odor and properly identify its intensity. Failure to receive a score of 100% meant a panelist would repeat the process. If the panelist failed to receive 100% after a second try the panelist was excused from the study. If a panelist received 100% on a second try, the panelist would be tested again to ensure mastery of discrimination.

3.1.5. *ADAPTATION*

Six subjects participated in the adaptation test after they successfully completed the training with all four odorants. Four different adaptation conditions were presented for each odorant tested. Each of the four odorants was tested three times for a total of 12 testing sessions. Each testing session lasted 45 minutes. Each adaptation condition was presented four times. Prior to each testing session panelists were asked to reacquaint themselves with the range of intensities of the references and the three references were present during all of the testing sessions.

The four adaptation conditions tested: control, scaling, self-adaptation, and cross-adaptation are outlined in Figure 3.3. Panelists were presented with three bottles during testing. The first and third bottles were rated for their

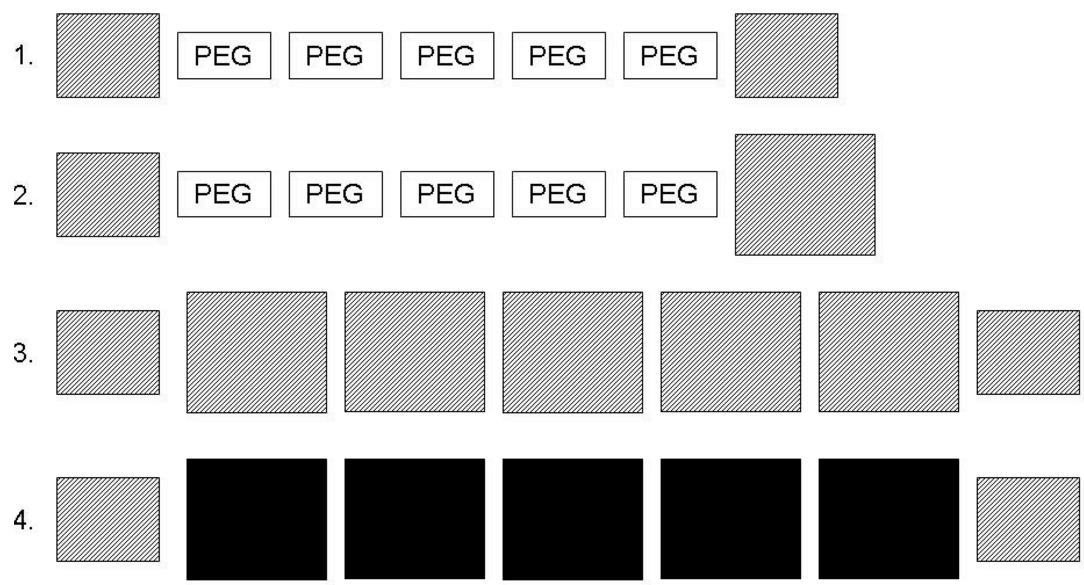


Figure 3.3: The adaptation scheme presented. Each box represents a sniff. The size of the box indicates the intensity of the odorant presented. The color of the box indicates the odorant type presented. Condition 1 is the control. The first bottle is a medium intensity of an odorant. PEG is the adapting stimulus and the final bottle is the medium intensity of the same odorant. Condition 2 is scaling. The purpose of this condition is to ensure the panelist uses the full range of the scale. The first and third bottles are different intensities. The first bottle is the medium intensity of an odorant, PEG is the adapting stimulus, and the final bottle is a high intensity of the same odorant presented in the first bottle. Condition 3 is self-adaptation: the first bottle is the medium intensity of an odorant. The adapting stimulus is high intensity of the same odorant; the third bottle is the medium intensity of the same odorant. Condition 4: Cross-adaptation. The first bottle is medium intensity of the odorant; the adapting stimulus is a high intensity of a different odorant. The third bottle is the medium intensity of the odorant presented in the first bottle.

intensity; the second bottle was used as the adapting stimulus. In the control condition: the first and third bottles contained the same odorant at the middle intensity and the adapting stimulus (bottle 2) was PEG (solvent). The scaling condition served to check whether the panelists were using the full-range of the perceived intensity scale. The first bottle contained a medium intensity of the odorant, the adapting stimulus contained PEG (solvent) and the third bottle contained a high intensity of the same odorant contained in the first bottle. For the third condition, self-adaptation, all three bottles contained the same odorant. However, the adapting stimulus contained a high intensity of the odorant tested, while the first and third bottles contained a medium intensity of the same odorant. The final condition was cross-adaptation. In this condition, the adapting stimulus (the second bottle) contained a different odorant than in the first and third bottles. Presentations of the conditions were randomized throughout testing.

Panelists used a 5-point scale to rate the intensity. Subject's evaluated three bottles. For each adaptation set, subjects were told that the first bottle in each set medium intensity. They were informed that the perceived intensity of the third bottle might be greater than, less than, or equal to the intensity of the first bottle. In order for panelists to indicate a change in perceived intensity it was suggested that they start in the middle of the scale. The purpose of the experiment was not to evaluate the intensity of the perception, but the direction of the change. Panelists were asked to alert the researcher if the first test bottle was too weak or too strong.

Panelists were presented with three bottles and copied the random three-digit code written on the bottle into the space allotted on the ballot (example shown in Figure 3.4). Panelists wrote the number of the second

Name:______________________

email:______________________

Please **write** the number of the bottle in the blank space and **rate** the intensity of the odor in the first bottle presented.
Take **FIVE** deep breaths each lasting three seconds from the second bottle you are handed.
Please **write** the number of the bottle in the blank space and **rate** the intensity of the odor in the third bottle.

Please rate the following solutions on a scale of 1-5 (1-no detectable odor, 5- very strong odor).
Circle the number which best fits your answer.

____

5

No odor   Slight odor   Medium odor   Slightly strong   Very Strong

No odor   Slight odor   Medium odor   Slightly strong   Very Strong

Figure 3.4: Adaptation ballot. Panelists evaluated the perceived intensity of the odorant before and after adaptation. The box on the left-hand side is where panelists wrote the number of the second bottle presented.

bottle in the box on the left-hand side of the ballot. During testing panelists rated their judgments using the scale. Prior to testing the researcher demonstrated the proper adaptation technique and asked the subject to mimic this technique in order to ensure proper stimulus delivery. Once the panelist smelled the first bottle and made an intensity judgment he or she would take five deep breathes from the second bottle (the adapting stimulus) and immediately follow with one more deep breath from third bottle and judge its intensity. Prior research has shown that an individual is capable of detecting and judging an odorant using a single sniff (Laing et al 1984). Once a panelist completed evaluating a set they took a two-minute break before the presentation of another odorant set to allow them to recover from the adaptation.

3.1.6 *Data Analysis*

Adaptation was determined as the mean difference between the rating of the first bottle and the rating of the third bottle. Therefore, the greater the difference between the rating of the first bottle and the third bottle, the larger the level of adaptation observed. Data were analyzed using a Mathematica 6.0 notebook. Means and confidence intervals were determined for the four conditions and the four odorants tested. The data were clustered into three groups: self-adaptation, green cross-adaptation which included any odorant cross-adapted with C_6, and citrus-odor cross-adaptation which included all combinations of C_8, C_{10}, and C_{11} citrus smelling odorants, graphed (Figure 3.5) and analyzed statistically (Table 3.2). Analysis of variance (ANOVA) was performed on the adaptation tests.

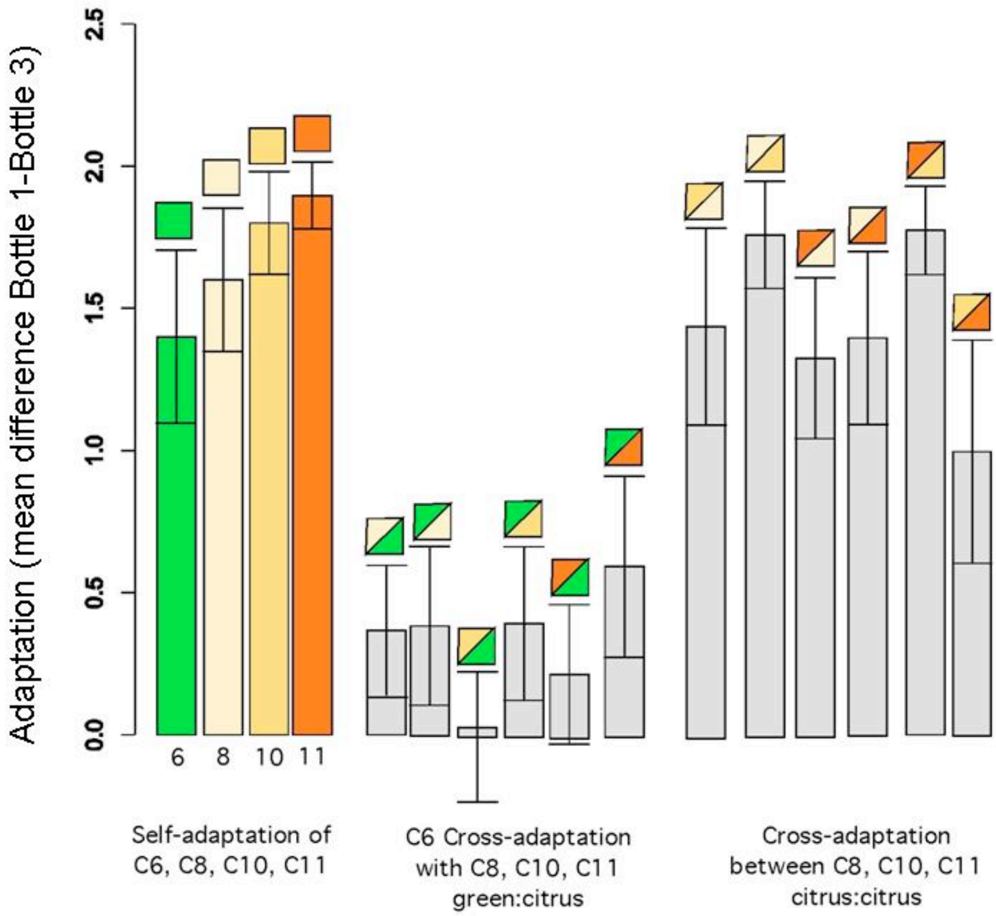


Figure 3.5: Results of adaptation. The color of the blocks indicates the odors involved in the adaptation. Where the upper left triangle is the adapting stimulus, the lower right triangle is the evaluating stimulus. The height of the bars indicates the amount of adaptation, the taller the bar the greater the adaptation. In the color scheme, green is C_6, off-white is C_8, tan is $C_{10,}$, and orange is C_{11}. The left most set labeled "self-adaptation" the evaluating and adapting stimulus are the same. The center-most set of blocks is cross-adaptation with C_6, The right most set of blocks labled 'cross-adaptation between C_8, C_{10}, and C_{11}. Self-adaptation is strong for all odorants. Cross-adaptation is strong between all sets of citrus odorants. Cross-adaptation with C_6 is low.

Table 3.2: Results table of all adaptation conditions tested with means and significance.

	Evaluating Stimulus/ Adaptation Stimulus	Mean	Significance LSD	Significance Tukey
Self-Adaptation	6/6	1.40	C	AB
	8/8	1.60	AB	A
	10/10	1.80	AB	A
	11/11	1.88	A	A
Hexanal Cross-Adaptation	6/8	0.36	EF	DE
	8/6	0.39	EF	CDE
	6/10	0.03	G	E
	10/6	0.40	EF	CDE
	6/11	0.23	FG	DE
	11/6	0.60	E	CD
Citrus Cross-Adaptation	8/10	1.45	BC	AB
	10/8	1.77	AB	A
	8/11	1.33	CD	AB
	11/8	1.40	C	AB
	10/11	1.00	D	BC
	11/10	1.77	AB	A

3.2 Results and Discussion

Previous electro-physiological data and free-choice profiling have outlined distinct odor quality differences as well as differences in OR binding between C_6 and C_8, C_{10}, C_{11}. Figure 3.5 divides the adaptation into three groups. The left-most group is self-adaptation, the center group represents odorants cross-adapted with C_6 and the right most set of bars represents citrus cross-adaptation (cross-adaptation of C_8, C_{10}, C_{11}). The height of the bars is a measure of adaptation calculated from the difference of the perceived intensity of bottle 1 minus the perceived intensity of bottle 3. As mentioned earlier, the larger the difference between the intensity rating for these bottles, the greater the level of adaptation observed. The colors in the figure represent the evaluation odorant (lower right triangle) and the adapting odorant (upper left triangle). As shown by the four bars on the all four odorants self-adapt to the same magnitude within 95% confidence limits, while the set of bars labeled “C6 cross-adaptation” show significantly less cross-adaptation than the self-adaptation for each odorant. The center set of bars show that none of the odorants cross-adapted with the green smelling C_6 as strongly as they self-adapt. However, the bars labeled “cross-adaptation between C_8, C_{10}, C_{11}” show significant cross-adaptation in both directions. These results mimic the findings in rats showing different receptor binding than C_6 as well as the descriptive analysis of these aldehydes in humans describing hexanal as having a different odor character and than C_8, C_{10}, C_{11}.

The mean values for self-adaptation and cross-adaptation are shown in Table 3.2. Two orthogonal contrasts were made between the means of the C_6 cross-adaptation group, and the joint means of the self-adaptation and citrus adaptations as well as between the mean self-adaptation and citrus adaptations. Significance of the contrasts were significant at $P<<0.0001$ and $P=0.01$ respectively. The F-statistic (1, 13) calculated for the contrast of the self-adaptation set and citrus cross-adaptation versus the C_6 cross-adaptation was 332.15 (p-value<<0.0001). The F-statistic (1, 465) calculated for the contrast between self-adaptation and citrus cross adaptation was 6.66 (p-value =0.01). Based on the least-significant difference (LSD) test among the adaptations all of the hexanal cross-adaptations differ significantly from the self-adaptation group and the citrus cross-adaptation group. The same is true for the Tukey test. Additionally based on the LSD and Tukey test there are differences between the self-adaptation and citrus cross-adaptation groups. However, some of the citrus cross-adaptations are not significantly different from the self-adaptation condition.

As predicted by the results of Zhao et. al. (1998) and Nagata (1990) there was a marked difference in cross-adaptation between C_6 and C_8, C_{10}, and C_{11} (Nagata & Takeuchi 1990, Zhao et al 1998). Self-adaptation is fairly uniform across all four odorants and as is the cross-adaptation between C_8, C_{10}, and C_{11}. In a pilot study, hexanal demonstrated lower self-adaptation than the other odorants but this difference disappeared when the intensity of the adapting stimulus was increased. Vaschide (1901) noted that some odorants do not self-adapt as effectively as other odorants, hexanal may be one of these odorants (Vaschide 1901). Perhaps hexanal's much higher detection threshold contributes to difficulties in self-adaptation.

Odorants with similar odorant characteristics have been shown to cross-adapt more readily than dissimilar odors (Cain & Polak 1992, Gottfried et al 2006, Todrank et al 1991). However, it should be noted that there are instances within the literature where structurally similar but perceptually distinct odorants demonstrated asymmetric cross-adaptation (Pierce et al 1996). It seems logical that odorants binding a common receptor site are more likely to cross-adapt symmetrically than odorants binding to different receptor sites (Dalton 2000, Kadohisa & Wilson 2006, Wilson 1998). A theory explaining cross-adaptation has been proposed by Vaszquez-Prado (2003) which suggests odorants cross-adapt because of cross-talk between receptor neurons. Hill (1998) and Vaszquez-Prado (2003) suggested that cross-talk occurred when one class of receptors alters the response of another receptor class. This has been witnessed in the C. Elegans (L'Etoile et al 2002) as well as in drosphilia (Boyle & Cobb 2005). This theory would suggest that the activation of a receptor by C_{10} would also signal the activation of ORs for C_{11}.

3.3 Conclusions

The findings show significantly less cross-adaptation with hexanal than octanal, decanal, and undecanal. It is possible that humans use different receptors to detect hexanal as it has been shown in rats. Furthermore, it is likely that octanal, decanal, and undecanal share a common set of receptors distinct from the receptors of hexanal, which could explain the differences in the green and citrus odor character of these odorants. This study found distinct differences between C_6 and the C_8, C_{10}, C_{11} odorants establishing a set of dissimilar odors that can be further examined in mixtures.

REFERENCES

Boyle J, Cobb M. 2005. Olfactory coding in Drosphila larvae investigated by cross-adaptation. *The Journal of Experimental Biology* 208: 3483-91

Cain W-S, Polak E-H. 1992. Olfactory adaptation as an aspect of odor similarity. *Chemical Senses*

Cometto-Muniz J-E, Cain W-S, Abraham M-H. 2005. Odor detection of single chemicals and binary mixtures. *Behavioral Brain Research*

Dalton P. 2000. Psychophysical and Behavioral Characteristics of Olfactory Adaptation. *Chemical Senses* 25: 487-92

Gottfried J-A, Winston J-S, Dolan R-J. 2006. Dissociable codes of odor quality and odorant structure in human piriform cortex. *Neuron*

Hill SM. 1998. Receptor crosstalk: Communication through cell signaling pathways. *Anat. Rec.* 253: 42-8

Huang YT, Lawless HT. 1998. Sensitivity of the ABX Discrimination Test. *Journal of Sensory Studies* 13: 229-39

Kadohisa M, Wilson D-A. 2006. Olfactory cortical adaptation facilitates detection of odors against background. *Journal of Neurophysiology (Bethesda)*

L'Etoile ND, Coburn CM, Eastha J, Kistler A, Gallegos G, Bargmann CI. 2002. The cyclic GMP-dependent protein kinase EGL-4 regulates olfactory adaptation in C. elegans. *Neuron* 36

Laing DG, Panhuber H, Willcox ME, Pittman EA. 1984. Quality And Intensity Of Binary Odor Mixtures. *Physiology and Behavior*

Lawless HT, Heymann HH. 1998. *Sensory Evaluation of Food*. New York: Springer

Macmillan NA, Creelman D. 2005. *Detection Theory: A Uster's Guide*. Mahwah, NJ: Lawrence Erlbaum Associates

Nagata Y, Takeuchi N. 1990. Determination of odor threshold value by triangle odor bag method. *Bulletin of Japan Environmental Sanitation Center* 17: 77-89

Pierce JD, Wysocki CJ, Aronov EV, Webb JB, Boden RM. 1996. The Role of Perceptual and Structural Similarity in Cross-adaptation. *Chemical Senses* 21: 223-37

Todrank J, Wysock CJ, Beauchamp GK. 1991. The Effects Of Adaptation On The Perception Of Similar And Dissimilar Odors. *Chemical Senses*

Vaschide N. 1901. L'experience de Wever et l'olfaction en milieu liquide. *Compt. rend. Soc. Biol.* 53

Vasquez-Prado J, Casas-Gonzalez P, Garcia-Sainz JA. 2003. G protein-coupled receptor cross-talk: pivotal roles of protein phosphorylation and protein-protein interactions. *Cell Signal* 15: 549-57

Wilson DA. 1998. Synaptic correlates of odor habituation in the rata anterior piriform cortex. *Journal of Neurophysiology (Bethesda)* 80: 998-1001

Zhao H, Ivic L, Otaki J-M, Hashimoto M, Mikoshiba K, Firestein S. 1998. Functional expression of a mammalian odorant receptor. *Science (Washington D C)*

CHAPTER 4

EXPERIMENT 2: REFERENCE MATCHING OF DISSIMILAR BINARY ODOR MIXTURES

4.1 MATERIALS & METHODS

4.1.1 *PANELISTS*

Six panelists, five women and one man, non-smokers with normal olfactory function and mean age of 28.2 (S.D. 3.2) years, volunteered to participate. Experimental protocol was reviewed and approved by the Institutional Review Board of Cornell University as listed in the Appendix (A.2). All subjects were paid for their participation.

4.1.2. *STIMULI*

Straight chain aliphatic aldehydes C_6 and C_8, were obtained from Sigma-Aldrich (ST. Louis, MO) as listed in the appendix. All odorants were dissolved in Poly(ethylene glycol) (PEG). Low (L), medium (M), and high (H) intensity dilutions were made for both odorants. L, M, and H intensities were determined by dose-response curves collected for Experiment 1. C_6 concentrations were 4.1 mM (6L), 20.3 mM (6M), 244 mM (6H) and C_8 were 1.0 mM (8L), 4.0 mM (8M), and 64.0 mM (8H) as listed in Table 4.1.

Odorants were presented in 250 mL polyethylene squeeze bottles modified with 1.5 cm diameter Teflon ball fitted around the neck of the bottle for nasal comfort, and labeled with random three-digit codes. A drill bit (0.2160 in. diameter) was used to make a hole in the center of the Teflon balls. Two odorants were present within each binary mixture C_6 (6) and C_8 (8) at one of the three levels (L,M,H). For example a mixture written as 6L-8M contained a low intensity of C_6 and a medium intensity of C_8. There were five binary mixture stimuli: 8M-6L, 8M-6M, 8M-6H, 6M-8L, 6M-8H. Stimuli were made by

Table 4.1. Stimuli concentrations of C_6 and C_8 in mM.

	L	M	H
C_6	4.1	20.3	244
C_8	1.0	4.0	64.0

dipping perfumer's blotters 1 cm in PEG solutions of the odorants, placing them in 250 mL poly(ethylene) plastic squeeze bottles, and allowing them to equilibrate for at least one hour prior to testing. All bottles contained two perfumer's strips. For single odorants, one strip was dipped in the PEG based odorant stimuli and the other in pure PEG.

4.1.3 *Training*

Six panelists were trained to distinguish L, M, and H intensities of each odor using a reference-matching task (ABC-X) for both C_6 and C_8 as described in Chapter 3. However, instead of the references being A (PEG), B (Medium Intensity), and C (High Intensity). The references were A (Low Intensity), B (Medium Intensity), and C (High Intensity). Panelists were trained in separate sessions for odorants C_6 and C_8. The AB-X sensory task asks panelists to match stimulus X to either reference A or B (Huang & Lawless 1998, Lawless & Heymann 1998). Panelists were trained in separate tasks to identify the intensities of hexanal and octanal.

For each odorant training session, panelists were presented with three intensity references of a single odorant A (L), B (M), and C (H). Once familiar with the three intensity references, subjects were presented with seven randomly placed bottles: two low intensity, two medium intensity, and two high intensity bottles. One extra bottle of low intensity, medium intensity, or high intensity was added to the sorting task in order to prevent process-of-elimination tactics. A 30-second break occurred between each test-bottle judgment. After the panelist sorted the seven bottles according to intensity the subject notified the researcher. The researcher checked the accuracy of the panelist's bottle placement. If there were errors in placement, the researcher alerted the panelist. After a 5-minute break the process was repeated with another set of seven bottles. In order for a panelist to proceed onto the mixture testing experiment, the panelist had to properly place 100% of the bottles in two subsequent tests. All six panelists continued onto the mixture tests.

4.1.4 *MIXTURE INTENSITY REFERENCE-MATCHING TASK*

Panelists were presented with the same intensity references A (Low), B (Medium), and C (High) as in the training session. However, the panelists evaluated nine bottles rather than seven bottles. Three of the bottles contained mixtures. A 30-second break took place between bottle-evaluation. Two groups were presented per testing session, with a five-minute break between groups.

In separate sessions, panelists were instructed to evaluate bottles for the intensity of hexanal or the intensity of octanal. If the reference bottles contained hexanal, the panelist was asked to identify the intensity of hexanal in the mixture (hexanal reference matching). If the reference bottles contained octanal, the subject was asked to identify the intensity of octanal within the test-bottle (octanal reference matching).

Nine different test-bottles were presented for evaluation during each of the reference matching sessions. Six of these bottles contained a single odorant: 2 bottles of low intensity, 2 bottles of medium intensity, and 2 bottles of high intensity. The single odorant was always the same as the odorant in the reference bottles. If the reference task were defined as a C_6 matching task, all bottles containing single odorants would contain C_6 (the same as in the training). Additionally three bottles containing binary mixtures were presented. Each mixture bottle contained two odorants: hexanal and octanal. One odor was defined as the figure (the odorant being evaluated) the other as the ground. The figure odor was always the same odor as the reference. Thus if the reference was hexanal, the subject would be asked to identify the intensity of hexanal within the mixture and try to ignore the intensity of octanal.

For example in a C_6 matching task if a subject were presented with a mixture of **6L**-8M (the figure is indicated in **bold**) the subject would identify the low intensity of C_6 within this binary mixture and hopefully match this to the 6L reference.

Figure 4.1 a and b illustrates the two mixture tasks tested: “Ground Constant” and “Figure Constant” and the types of mixtures presented in both tasks. These graphs only illustrate the tasks performed when the reference is designated as C_6. However, the inverse experiment, where C_8 is the reference was also performed. In this figure the graph labeled “Ground Constant” illustrates the case when the figure odorant (C_6) increased from low to high while the ground intensity, C_8, is held constant at a medium intensity. In this task bottles containing 6L-8M, 6M-8M, 6H-8M were presented, where the panelist identified the intensity of C_6 in these bottles. The graph labeled “Figure Constant” illustrates the mixtures presented when the figure (C_6) was held constant in a C_6 matching task. In this condition the intensity of octanal (ground) increases from low to high in the bottles, while hexanal (figure) is always medium. These mixtures were 6M-8L, 6M-8M, 6M-8H.

Figures 4.2a and 4.2b illustrate the tasks performed during single task (C_6 matching task). Figure 4.2a is an illustration of a C_6 matching task when the figure is varied and the ground is constant. The dashed line is indicative of a bottle placed in front a reference of different intensity than the figure intensity within the bottle. Figure 4.2b illustrates a C_6 matching task when the figure is constant and the ground is varied.

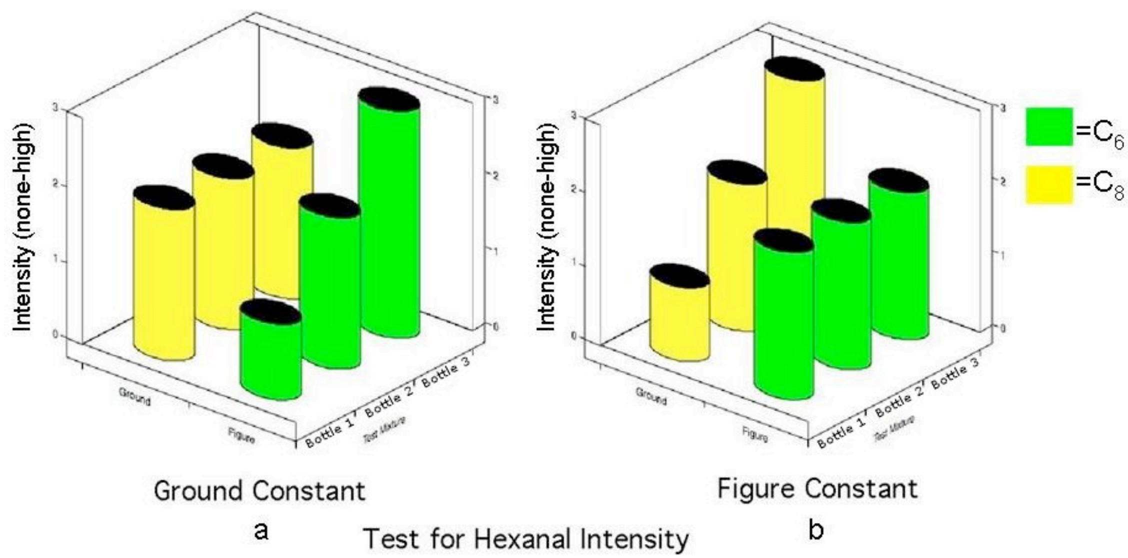


Figure 4.1a and b: Experimental design testing hexanal intensity, hexanal is the figure. Green bars are C_6 and yellow bars are C_8. The Y-axis labeled 0-3, is the level of intensity. 0=none, 1=low, 2=medium, 3=high. The test-mixtures are illustrated as a combination of C_6 (figure) and C_8 (ground). In the graph labeled "Ground Constant," the intensity of C_6 increases from low to high. The intensity of C_8 is medium in every bottle. In the graph labeled "Figure Constant," the intensity of the figure C_6, is medium in all three mixtures, while the intensity of the ground (octanal) increases from low to high.

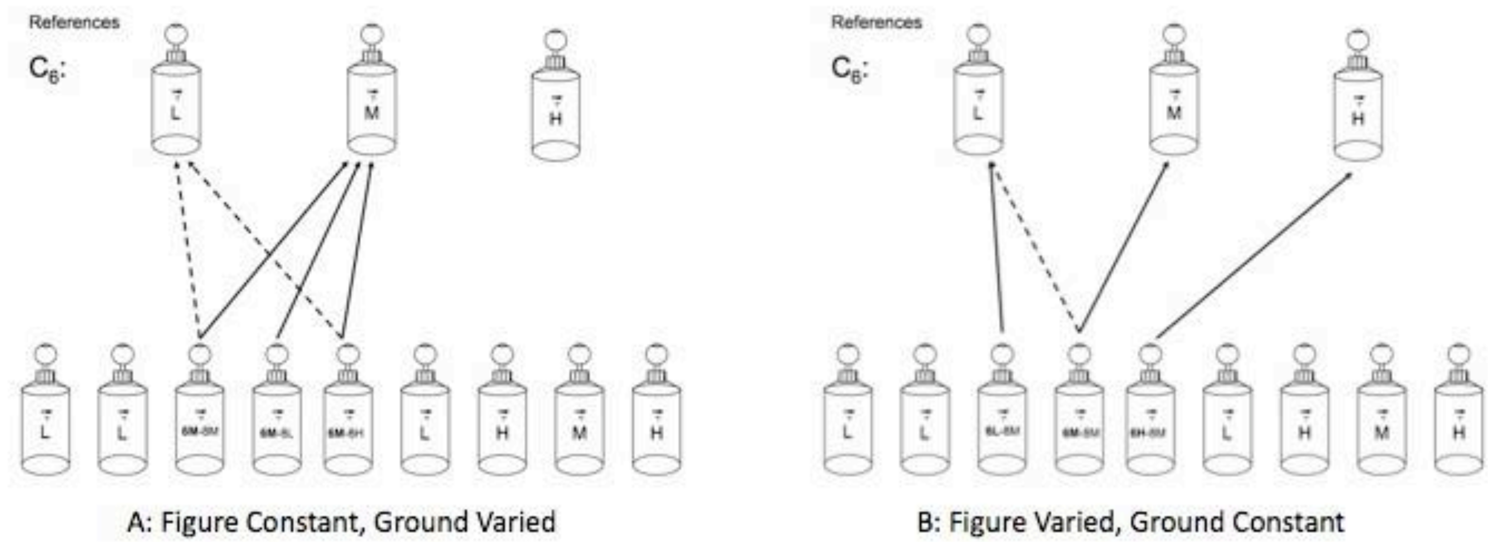


Figure 4.2a and b: Both images illustrate a mixture reference task for hexanal. Diagram A: illustrates a figure-constant-ground varied task. Where hexanal the level of hexanal is M in the mixture bottles and octanal varies. Dashed arrows represent bottles that are placed in front of a lower intensity reference lower than that of the figure intensity in the bottle. Graphic B: Figure varied, ground constant contains mixtures where the

During evaluation panelists were asked whether the odorant smelled green, citrus, or neither. Panelists indicated that the two odorants were present within the binary mixture, with no other emergent odorants present within the mixtures.

4.1.5 Data Analysis

Data were scored according to the intensity of the figure and ground within the mixture as well as the placement of the test-bottle in front of the reference. The figure intensity was low, medium or high thus low=1, medium= 2, high =3. The ground odorants varied from nothing to high, nothing = 0, low = 1, medium = 2, high = 3. For single odorant, the ground PEG, this represented a condition where the ground=0 After the subjects completed their test-bottle evaluation, the researcher recorded the placement of the bottles in front of the references. The placement of the test-bottle was recorded, as a placement score: the test bottle could be placed in front the low reference=1, medium reference=2, high reference=3. Figure suppression was defined as the difference between the figure intensity and the placement score. Thus if a test-bottle containing 6M were placed in front of the 6M reference, the test bottle would be coded as figure=1, placement =1. The total score for this evaluation would =0 (Figure intensity – Placement Score). Similarly if in a C_8 matching task, mixture 8M-6L were placed next to 8M the score would equal 0. However, if 8M-6L were placed next to reference 8L the score would equal 1. The average of the difference in the placement score and figure intensity were plotted in Figures 4.3 and 4.4 As mentioned earlier a total of four evaluations were made per mixture per reference task, thus if all judgments were placed in front a reference of lower intensity than the figure intensity the total score would equal one.

Data were split into two separate groups. One group was defined as figure constant - ground varied condition and the other group was defined as the figure varied - ground constant condition. Each group included data from both C_6 and C_8 matching tasks. Both the figure constant - ground varied condition and the figure varied - ground constant conditions were analyzed through one-way ANOVAs with LSD and Tukey's test for significance. The data for both test conditions are presented in Tables 4.2 and 4.3. These data are also represented in Figure 4.3 and Figure 4.4.

4.2 RESULTS AND DISCUSSION

Panelists were required to evaluate the intensity of odorants within the binary mixture of green/ grassy C_6 and citrus C_8. Panelists were trained to identify the two different odorants and their respective intensities. The odorant panelists were required to identify within the mixture was designated as the figure odorant. The subject used a reference of the same odorant as the figure to match the intensity of the odorant in the test bottle. In binary mixtures, the other odorant present was the ground odorant. Results are illustrated in Figures 4.3 and 4.4. The two colors indicate the type of reference task. Yellow indicates, a C_8 reference matching task, and green represents a C_6 reference matching task. The Y-axis measures figure-suppression, the difference between figure score and placement score. The height of the bars indicates the level of figure suppression. Figure 4.3 illustrates the effect of figure suppression when the figure is held constant and the ground is varied. This graph indicates that as the ground intensity increases from low to medium, the intensity of the figure is increasingly obscured. When the figure intensity is

Table 4.2: Results for figure-constant, ground varied tasks.

	Mixture	Mean	Significance LSD	Significance Tukey
C_8 Task	8M-6L	0.13	D	D
	8M-6M	0.58	BC	ABC
	8M-6H	0.92	A	A
C_6 Task	6M-8L	0.25	D	D
	6M-8M	0.50	C	BC
	6M-8H	0.79	AB	AB

Table 4.3: Results for figure-varied, ground constant tasks.

	Mixture	Mean	Significance LSD	Significance Tukey
C_8 Task	8L-6M	-0.08	BC	B
	8M-6M	0.58	A	A
	8H-6M	0.13	B	B
C_6 Task	6L-8M	-0.20	C	B
	6M-8M	0.54	A	A
	6H-8M	0.08	B	B

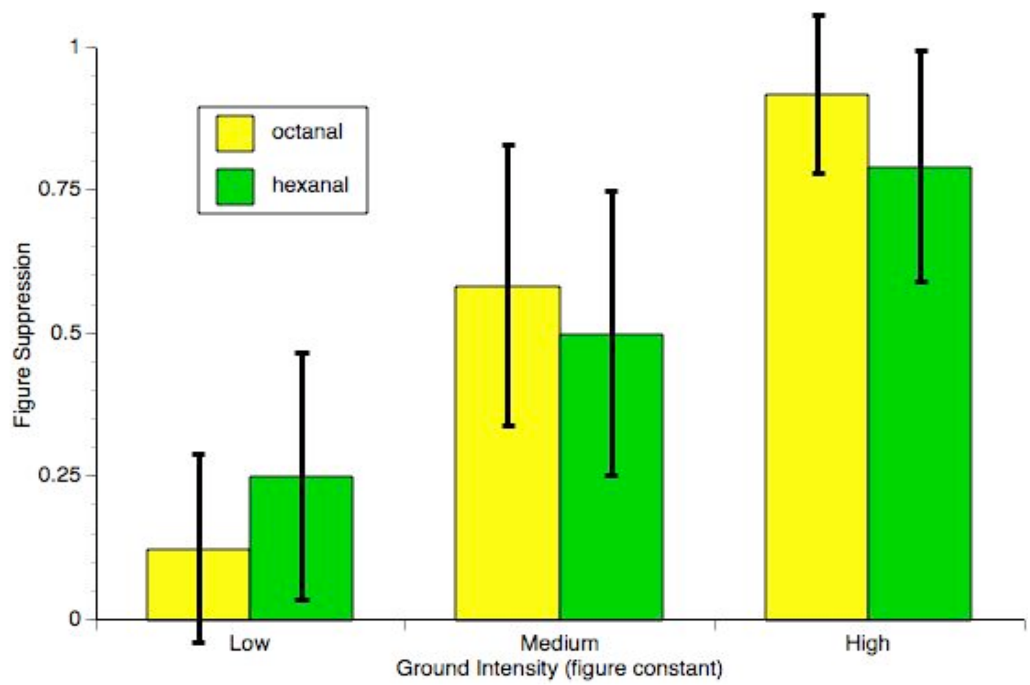


Figure 4.3: Results of Figure Constant – Ground Varied matching task with 95% confidence intervals. The yellow bars represent when octanal is the figure. The green bars represent when hexanal is the figure. The greater the height of the bars the more figure suppression. As the intensity of the ground increases figure suppression increases. Figure suppression is measured by the difference between the figure intensity and placement score.

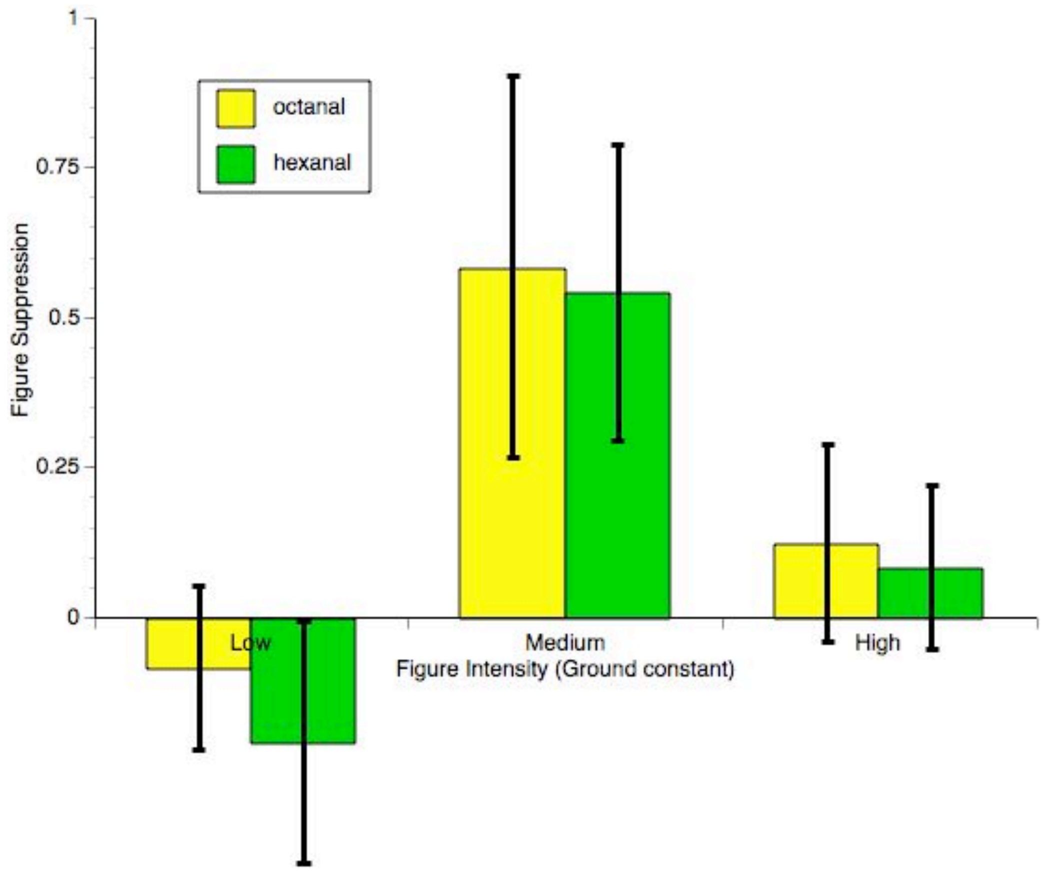


Figure 4.4: Results of figure varied – ground constant test with 95% confidence intervals. Yellow bars represent when octanal is the figure, green bars represent when hexanal is the figure. The height of the bars is the level of figure suppression. Figure suppression is Figure Intensity – Placement Score. As the figure intensity increases, there is less figure suppression.

medium and the ground intensity is high, the mixture is almost always placed next to a reference of lower intensity. As indicated in the Fig. 4.3 according to the ANOVA there were significant differences between the means of the different mixtures in the two reference conditions at α=0.01. $F(5,138) = 12.42$ (p-value<<0.001). Additionally as seen in Table 4.2 there are significant effects, as illustrated by both Tukey's HSD and LSD, of varing the ground concentration on the perception of the figure intensity. As illustrated in Figure 4.4 when the figure and ground are iso-intense there is a high level of figure suppression. Furthermore, when the figure intensity is of greater intensity than the ground, there is little error in properly placing the high intensity figure next to the high intensity reference. According to the ANOVA there are significant differences between the means of all six mixtures presented at α=0.01. $F(5, 138) = 13.37$ (p-value<<0.001). As illustrated in Table 4.3, both LSD and Tukey's HSD revealed a significant difference between each of the mixture treatments. Both iso-intense mixtures were perceived equally.

The two most widely accepted views of odor mixture perception argue that either the perception of mixtures is an elemental process, where each component within the mixture is detectable or configural, where the component odors combine to create a novel odorant (Laing & Wilcox 1983, Zhao et al 1998). The results from this experiment support elemental processing for these two odorants. Although research has demonstrated that mixtures create a novel perception, the components are still detectible within the mixture (Boyle et al 2008).

Although these results demonstrate mixture suppression of C_6 and C_8 in a binary mixture, Experiment 1 demonstrates little cross-adaptation between these two odorants. The inability to cross-adapt C_6 and C_8, combined with the known differences in activation of OR-I7 in the rat, and qualitative differences demonstrated through free-choice profiling (Kittel et al 2008), suggest these two odorants may excite different OR families. However, these two odorants display mixture suppression, suggesting that mixture suppression and cross-adaptation occur through different neural mechanisms. Further investigation examining the differences between mixture suppression and cross-adaptation is necessary.

It is understood that dissimilar odorants facilitate the identification of component odors within a mixture; however, depending upon the perceived intensity ratio of the odorants within the mixture, the intensity of a figure odorant can become increasingly more difficult to identify due to mixture suppression (Dalton 2000, Livermore & Laing 1998). Future research should examine similar odors within mixtures to see how the ratio of the odorant intensities within a mixture influences the degree of mixture suppression.

4.3 CONCLUSIONS

When there are distinct intensity differences between the two odorants in the binary mixtures the figure and the ground are recognizable. However, when the intensities are similar the distinctions become blurred, resulting in mixture suppression. This blurring can be compared to the ambiguous images in visual gestalt, where the figure and ground assignments are unclear. Thus the identification of the intensity of the individual odorants in these mixtures is more difficult even though the presence of both is apparent. The clear display of mixture suppression in this experiment suggests cross-adaptation and mixture suppression occur through different neural processes.

REFERENCES

Boyle JA, Djordjevic J, Olsson M-J, Lundstrom J, Jones-Gotman M. 2008. The Human Brain Distinguishes between Single Odorants and Binary Mixtures. *Cerebral Cortex*: 1-6

Dalton P. 2000. Psychophysical and Behavioral Characteristics of Olfactory Adaptation. *Chemical Senses* 25: 487-92

Huang YT, Lawless HT. 1998. Sensitivity of the ABX Discrimination Test. *Journal of Sensory Studies* 13: 229-39

Kittel KM, Barnard J, Kurtz AJ, Acree TE. 2008. Free-Choice Profiling of OR-I7 Agonists and Homologues Using GC-O. *Chemosensory Perception*: 1-15

Laing DG, Wilcox ME. 1983. Perception Of Components In Binary Odor Mixtures. *Chemical Senses*

Lawless HT, Heymann HH. 1998. *Sensory Evaluation of Food*. New York: Springer

Livermore A, Laing D-G. 1998. The influence of odor type on the discrimination and identification of odorants in multicomponent odor mixtures. *Physiology and Behavior*

Zhao H, Ivic L, Otaki J-M, Hashimoto M, Mikoshiba K, Firestein S. 1998. Functional expression of a mammalian odorant receptor. *Science (Washington D C)*

CHAPTER 5

CONCLUSION

The two experiments presented in this thesis reveal differences in the processing of odor information between the green smelling C_6 and the citrus smelling C_8, C_{10}, C_{11}. Highlighted in Experiment 1 is the low cross-adaptation of C_6 with the three other aldehydes. These psychophysical results closely parallel results from physiological and free-choice profiling studies of these odorants. Although C_{11} does not show activation of OR-I7 in rats in electrophysiological recordings, cross-adaptation does occur in humans between C_8, C_{10}, C_{11} suggesting these odorants may share a common citrus OR. Grassy/ green C_6 most likely signals an OR different from the other three odorants. Although C_6 and C_8 showed no cross-adaptation, in the second experiment C_6 and C_8 displayed mixture suppression when at equal intensity and when the figure odorant was of lesser intensity than the ground odorant.

Experiment 2 highlighted the ability of individuals to accurately match odorant intensities within a mixture to a reference. The results, demonstrated that an individual is capable of distinguishing components within a mixture when the figure odorant is of greater intensity than the ground odorant. As the intensity of the ground odorant approached and surpassed the intensity of the figure it was still recognizable but weakened. Furthermore, when the figure odorant was greater than the ground odorant the figure was consistently matched to the reference of equal intensity to the figure. The ability of individuals to identify the dissimilar odorants of C_6 and C_8 within binary mixtures was an easier task than the identification of similar odors in mixtures. Future studies should examine similar odorants in mixtures, to determine whether individuals are capable of accurately evaluating intensities of similar odorants in a mixture solution. Furthermore the mixtures of C_8 and C_{10} should be evaluated to determine whether odorants which share a similar odor quality, a common OR, and cross-adapt are capable of identification within a mixture. Studies examining mixtures of greater complexity with these odorants using three or four components will allow for a more revealing examination of odor mixture perception which will be valuable for future research in comprehending odor mixture processing.

APPENDIX

1. Human Subjects Consent form for Experiment 1.

Olfactory Mixture Perception Study Consent Form

You are invited to participate in a research study of olfactory mixture perception. You were selected as a possible participant because you have no factors that would contribute to general olfactory dysfunction; i.e. you likely have a normal sense of smell. We ask that you read this form and ask any questions you may have before agreeing to be in the study.

Background: Anecdotal reports of variation in odor quality with odorant mixtures have been made by flavorists and perfumers for years but mostly without supporting quantitative data. Olfactory researchers have examined the behavior of odorants in mixtures but have little consensus on how the individual perceives mixtures. The objective is to conduct mixture perception study on a particular family of odorants (hexanal, octanal, decanal, and undecanal) to better understand the individual odor characteristics as well as the effects of adaptation.

Procedures: If you agree to be in this study, we will ask you to do the following: Subjects will be asked to smell a series of odorants presented in squeeze bottles, containing perfume blotters that were soaked in their respective odorant. Odorants include Hexanal, Octanal, Decanal and

Undecanal. Each series will take 20 minutes. There are a total of **6** sessions, each lasting 20 minutes including training.

Risks and Benefits of Being in the Study: We do not anticipate any risks for you participating in this study, other than those encountered in day-to-day life. All odorants are found naturally in foods and presented at levels at or below levels found in foods. Any foods containing cooked lipids naturally contain these odorants. Safety data in the form of Material Data Safety Sheets (MSDS) are available at your request in the lab. There are no direct benefits to participation in this study. Indirect benefits include contribution to the advancement of our understanding of olfactory function and phenomic coding of olfaction.

Compensation: You will receive no compensation for your participation. If you are a student, no class credit is involved.

Voluntary Nature of Participation: Your decision whether or not to participate will not affect your current or future relations with Cornell University or with the experimenter (Terry Acree) in any way. If you decide to participate, you are free to withdraw at any time without affecting those relationships. Your name may be associated with the data collected.

Contacts and Questions: The researcher conducting this study is Terry Acree, assisted by Anne Kurtz. **<u>Please ask any questions you have now.</u>** If you have questions later, you may contact them at 351-787-2240, Food Science Building, NYSAES-Cornell University, Geneva, NY 14456, tea2@cornell.edu. Note that email communication may not be secure for confidentiality. If you have any questions or concerns regarding your rights as a subject in this study, you may contact the University Committee on Human Subjects (UCHS) at 607-255-5138, or access their website at http://www.osp.cornell.edu/compliance/UCHS/homepageUCHS.htm.

You will be given a copy of this form to keep for your records.

Statement of Consent: By signing below, I indicate that I am participating in this study voluntarily. I have read the above information, and have received answers to any questions I asked. I also indicate that to the best of my knowledge, my sense of smell is not impaired or compromised in any way, whether due to illness, injury, drug use (prescription or otherwise), past surgery, or any other cause and that I am not to the best of my knowledge pregnant or breast feeding. Also, I will not use any perfumes, scented body lotions or soaps on test days. All my questions about the experiment have been answered to my satisfaction. I am between the ages of 18 and 60, inclusive. I consent to participate in the study.

Name (Print) ________________________________ Date __________

Signature ___________________________________ Age___________

Circle One

Male Female

Circle One

Smoker Non-Smoker

2. Human Subjects Consent Form for Experiment 2.

Olfactory Mixture Perception Study Consent Form

You are invited to participate in a research study of olfactory mixture perception. You were selected as a possible participant because you have no factors that would contribute to general olfactory dysfunction; i.e. you likely have a normal sense of smell. We ask that you read this form and ask any questions you may have before agreeing to be in the study.

Background: Anecdotal reports of variation in odor quality with odorant mixtures have been made by flavorists and perfumers for years but mostly without supporting quantitative data. Olfactory researchers have examined the behavior of odorants in mixtures but have little consensus on how the individual perceives mixtures. The objective is to conduct mixture perception study on a particular family of odorants (hexanal, octanal, decanal, and undecanal) to better understand the individual odor characteristics as well as the effects of adaptation.

Procedures: If you agree to be in this study, we will ask you to do the following: Subjects will be asked to smell a series of odorants presented in squeeze bottles, containing perfume blotters that were soaked in their respective odorant. Odorants include Hexanal, Octanal, Decanal and Undecanal. Each series will take 20 minutes. There are a total of **6** sessions, each lasting 20 minutes including training.

Risks and Benefits of Being in the Study: We do not anticipate any risks for you participating in this study, other than those encountered in day-to-day life. All odorants are found naturally in foods and presented at levels at or below levels found in foods. Any foods containing cooked lipids naturally contain these odorants. Safety data in the form of Material Data Safety Sheets (MSDS) are available at your request in the lab. There are no direct benefits to participation in this study. Indirect benefits include contribution to the advancement of our understanding of olfactory function and phenomic coding of olfaction.

Compensation: You will receive no compensation for your participation. If you are a student, no class credit is involved.

Voluntary Nature of Participation: Your decision whether or not to participate will not affect your current or future relations with Cornell University or with the experimenter (Terry Acree) in any way. If you decide to participate, you are free to withdraw at any time without affecting those relationships. Your name may be associated with the data collected.

Contacts and Questions: The researcher conducting this study is Terry Acree, assisted by Anne Kurtz. **Please ask any questions you have now.** If you have questions later, you may contact them at 351-787-2240, Food Science Building, NYSAES-Cornell University, Geneva, NY 14456, tea2@cornell.edu. Note that email communication may not be secure for confidentiality. If you have any questions or concerns regarding your rights as a subject in this study, you may contact the University Committee on Human

Subjects (UCHS) at 607-255-5138, or access their website at http://www.osp.cornell.edu/compliance/UCHS/homepageUCHS.htm.

You will be given a copy of this form to keep for your records.

Statement of Consent: By signing below, I indicate that I am participating in this study voluntarily. I have read the above information, and have received answers to any questions I asked. I also indicate that to the best of my knowledge, my sense of smell is not impaired or compromised in any way, whether due to illness, injury, drug use (prescription or otherwise), past surgery, or any other cause and that I am not to the best of my knowledge pregnant or breast feeding. Also, I will not use any perfumes, scented body lotions or soaps on test days. All my questions about the experiment have been answered to my satisfaction. I am between the ages of 18 and 60, inclusive. I consent to participate in the study.

Name (Print) _________________________________ Date __________

Signature ____________________________________ Age___________

Circle One

Male Female

Circle One

Smoker Non-Smoker

3. Table of Compounds Used in Experiments 1 and 2

Table A.1: Compounds grade and CAS # used in experiments 1

	Grade	CAS#
C_6	98%	66-25-1
C_8	99%	124-13-0
C_{10}	98%	112-31-2
C_{11}	97%	112-44-7
Poly (ethylene glycol)	--	25322-68-3

* All compounds were reagent grade